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<b>(21) International Application Number:</b> PCT/US96/09469 <b>(22) International Filing Date:</b> 6 June 1996 (06.06.96) <b>(30) Priority Data:</b> 08/484,135 7 June 1995 (07.06.95) US <b>(71) Applicant (for all designated States except US):</b> JOHNSON & JOHNSON [US/US]; One Johnson & Johnson Plaza, New Brunswick, NJ 08933-0001 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> JOHNSON, Dana, L. [US/US]; 1343 Lonely Cottage Road, Upper Black Eddy, PA 18972 (US). ZIVIN, Robert, A. [US/US]; 6 Glenbrook Court, Lawrenceville, NJ 08648 (US). <b>(74) Agents:</b> CIAMPORCERO, Audley, A., Jr. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933-0001 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> AGONIST PEPTIDE DIMERS  <b>(57) Abstract</b>  The present invention is directed to the dimerization of agonists and antagonists of cell surface receptors and particularly to peptide dimers which behave as cell surface receptor agonists in their dimeric form. Such receptors belong to the dimerization-mediated activation class often observed among receptors for growth and differentiation factors. The agonists of this class of receptors is understood to effect dimerization of the receptor and thus signal initiation. The present invention exemplifies dimers of erythropoietin (EPO) agonists and antagonists comprising a core amino acid sequence of X <sub>3</sub> X <sub>4</sub> X <sub>5</sub> GPX <sub>6</sub> TWX <sub>7</sub> X <sub>8</sub> (SEQ ID NO: 1) wherein each amino acid is indicated by standard one letter abbreviation; X <sub>3</sub> can be C, A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc; X <sub>4</sub> can be R, H, L or W; X <sub>5</sub> can be M, F, or I; X <sub>6</sub> is independently selected from any one of the 20 genetically coded L-amino acids or the stereoisomeric D-amino acids; X <sub>7</sub> can be D, E, I, L or V; and X <sub>8</sub> can be C, A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc, provided that either X <sub>3</sub> or X <sub>8</sub> is C or Hoc.		

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## AGONIST PEPTIDE DIMERS

### FIELD OF THE INVENTION

The present invention is directed to the dimerization of agonists and antagonists of cell surface receptors and particularly to peptide dimers which behave as cell surface receptor agonists in their dimeric form. Such receptors belong to the dimerization-mediated activation class often observed among receptors for growth and differentiation factors. The agonists of this class of receptors is understood to effect dimerization of the receptor and thus signal initiation. The present invention exemplifies dimers of erythropoietin (EPO) agonists and antagonists comprising a core amino acid sequence of  $X_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 1) wherein each amino acid is indicated by standard one letter abbreviation;  $X_3$  can be C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc;  $X_4$  can be R, H, L or W;  $X_5$  can be M, F, or I;  $X_6$  is independently selected from any one of the 20 genetically coded L-amino acids or the stereoisomeric D-amino acids;  $X_7$  can be D, E, I, L or V; and  $X_8$  can be C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc, provided that either  $X_3$  or  $X_8$  is C or Hoc.

### BACKGROUND OF THE INVENTION

Erythropoietin (EPO) is a glycoprotein hormone with an approximate molecular weight of 34,000 daltons. The primary role of EPO, which is synthesized in the kidneys of mammals, is to stimulate mitotic cell division and differentiation of erythrocyte precursor cells. As a result, EPO acts to stimulate and to

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1 regulate the production of erythrocytes. Erythrocytes,  
and the hemoglobin contained therein, play a central  
role in supplying oxygen to the body. Thus, the  
stimulation of erythrocyte production is able to  
5 increase the oxygen-carrying capacity of the blood.

During normal conditions, EPO is present in  
very low concentrations in plasma. Under hypoxic  
conditions, the amount of EPO in the circulation is  
increased in response to reduced O<sub>2</sub> blood levels.  
10 Hypoxia may be caused from various conditions including  
the loss of large amounts of blood, destruction of red  
blood cells by over-exposure to radiation or  
chemotherapeutic agents, reduction in oxygen intake due  
to high altitudes or prolonged unconsciousness, or by  
15 various forms of anemia. As the hypoxic condition  
diminishes, the amount of EPO produced subsequently  
diminishes.

Because of the essential role of EPO in red  
blood cell formation, the hormone is useful in both the  
20 diagnosis and the treatment of blood disorders  
characterized by low or defective red blood cell  
production. Recent studies provide a basis for the  
efficacy of EPO therapy in a variety of disease states,  
disorders, and states of hematologic irregularity,  
25 including: beta-thalassemia (See, Vedovato et al.  
(1984) Acta. Haematol. 71:211-213); cystic fibrosis  
(See, Vichinsky et al. (1984) J. Pediatric 105:15-21);  
pregnancy and menstrual disorders (See, Cotes et al.  
(1983) Brit. J. Obstet. Gynecol. 90:304-311); early  
30 anemia of prematurity (See, Haga et al. (1983) Acta  
Pediatr. Scand. 72:827-831); spinal cord injury (See,

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1 Claus-Walker et al. (1984) Arch. Phys. Med. Rehabil.  
65:370-374); space flight (See, Dunn et al. (1984) Eur.  
5 J. Appl. Physiol. 52:178-182); acute blood loss (See,  
Miller et al. (1982) Brit. J. Haematol. 52:545-590);  
aging (See, Udupa et al. (1984) J. Lab. Clin. Med.  
103:574-588); various neoplastic disease states  
accompanied by abnormal erythropoiesis (See, Dainiak et  
al. (1983) Cancer 5:1101-1106); and renal insufficiency  
(See, Eschbach et al. (1987) N. Eng. J. Med. 316:73-78).

Although purified, homogenous EPO has been  
characterized, little is known about the mechanism of  
EPO-induced erythroblast proliferation and  
differentiation. The specific interaction of EPO with  
15 progenitor cells of immature red blood cells, platelets,  
and megakaryocytes has not been described. This is due  
in part, to the small number of surface EPO receptor  
molecules on normal erythroblasts and on the  
erythroleukemia cell lines. See Krantz and Goldwasser  
(1984) Proc. Natl. Acad. Sci. USA, 81:7574-7578; Branch  
20 et al. (1987) Blood 69:1782-1785; Mayeux et al. (1987)  
FEBS Letters 211:229-223; Mufson and Gesner (1987) Blood  
69:1485-1490; Sakaguchi et al. (1987) Biochem. Biophys.  
Res. Commun. 146:7-12; Sawyer et al. (1987) Proc. Natl.  
25 Acad. Sci. USA 84:3690-3694; Sawyer et al. (1987) J.  
Biol. Chem. 262:5554-5562; and Todokoro et al. (1988)  
Proc. Natl. Acad. Sci. USA 84:4126-4130. The DNA  
sequences and encoded peptide sequences for murine and  
human EPO receptor proteins have been described. See,  
30 D'Andrea et al. PCT Patent Publication No. WO 90/08822  
(published 1990).

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1           The EPO-receptor (EPO-R) belongs to the class  
of growth-factor-type receptors which are activated by a  
ligand-induced protein dimerization. Other hormones and  
5           cytokines such as human growth hormone (hGH),  
granulocyte colony stimulating factor (G-CSF), epidermal  
growth factor (EGF) and insulin can cross-link two  
receptors resulting in juxtaposition of two cytoplasmic  
tails. Many of these dimerization-activated receptors  
10           have protein kinase domains within the cytoplasmic tails  
that phosphorylate the neighboring tail upon  
dimerization. While some cytoplasmic tails lack  
intrinsic kinase activity, these function by association  
with protein kinases. The EPO receptor is of the latter  
15           type. In each case, phosphorylation results in the  
activation of a signaling pathway.

          In accordance with the present invention, it  
has been discovered that the dimerization of peptide  
agonists and antagonists of dimerization-mediated  
20           receptors, such as EPO-R, increase the biological  
efficacy relative to the biological activity of the  
'monomeric' agonists and alters the properties of the  
antagonists such that, these dimers function as  
agonists, exhibiting biological activity.

#### 25           SUMMARY OF THE INVENTION

          In a first embodiment, the present invention  
is directed to peptide dimers which behave as cell-  
surface receptor agonists, dimers which exhibit binding  
and signal initiation of growth factor-type receptors.  
30           In one embodiment, the present invention provides  
peptide dimers which behave as EPO agonists. These

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1 dimers have two 'monomeric' peptide units of 10 to 40 or  
more amino acids, preferably 14 to about 20 residues in  
length, comprising a core amino acid sequence of  
5  $X_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 1) where each amino acid is  
indicated by standard one letter abbreviation;  $X_3$  can be  
C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid, or Hoc, where Hoc is  
homocysteine;  $X_4$  can be R, H, L, or W;  $X_5$  can be M, F,  
or I;  $X_6$  is independently selected from any one of the  
10 20 genetically coded L-amino acids or the stereoisomeric  
D-amino acids;  $X_7$  can be D, E, I, L, or V; and  $X_8$  can be  
C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid, or Hoc, where Hoc is  
homocysteine, provided that either  $X_3$  or  $X_8$  is C or Hoc.  
Preferably, the monomeric peptide unit of the dimer  
15 comprises a core sequence  $YX_2X_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID  
NO: 2) where each amino acid is indicated by standard  
one letter abbreviation; each  $X_2$  and  $X_6$  is independently  
selected from any one of the 20 genetically coded L-  
amino acids;  $X_3$  can be C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric  
acid, or Hoc, where Hoc is homocysteine;  $X_4$  can be R, H,  
20 L, or W;  $X_5$  can be M, F, or I;  $X_7$  can be D, E, I, L, or  
V; and  $X_8$  can be C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid, or  
Hoc, where Hoc is homocysteine, provided that either  $X_3$   
or  $X_8$  is C or Hoc.

25 More preferably, the monomeric peptide unit of  
the dimer comprises a core sequence of amino acids  
 $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$  (SEQ ID NO: 3), where each  
amino acid is indicated by standard one letter  
abbreviation; each  $X_1$ ,  $X_2$ ,  $X_6$ ,  $X_9$ ,  $X_{10}$ , and  $X_{11}$  is  
independently selected from any one of the 20  
30 genetically coded L-amino acids;  $X_3$  can be C, A,  $\alpha$ -  
amino- $\gamma$ -bromobutyric acid, or Hoc, where Hoc is

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1 homocysteine;  $X_4$  can be R, H, L, or W;  $X_5$  can be M, F, or I;  $X_7$  can be D, E, I, L or V; and  $X_8$  can be C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid, or Hoc, where Hoc is  
 5 homocysteine, provided that either  $X_3$  or  $X_6$  is C or Hoc.

In a more preferred embodiment, both  $X_3$  and  $X_6$  are C and thus, the monomeric peptide unit of the dimer comprises a core sequence of amino acids  
 $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$  (SEQ ID NO: 4). More preferably, the monomeric peptide unit comprises a core sequence of amino acids  $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$  (SEQ ID NO: 5), where  $X_4$  can be R or H;  $X_5$  can be F or M;  $X_6$  can be I, L, T, M, or V;  $X_7$  is D or V;  $X_9$  can be G, K, L, Q, R, S, or T; and  $X_{10}$  can be A, G, P, R, or Y. In a most preferred embodiment, the monomeric peptide unit of  
 15 the dimer will comprise a core sequence of amino acids  $X_1YX_2CX_4X_5GPX_6TWX_7CX_9X_{10}X_{11}$  (SEQ ID NO: 6), where  $X_1$  can be D, E, L, N, S, T, or V;  $X_2$  can be A, H, K, L, M, S, or T;  $X_4$  is R or H;  $X_5$  can be K, R, S, or T; and  $X_{10}$  is P. Particularly preferred monomeric peptide units of  
 20 the dimers include:

	GGLYLCRFGPVTWDCGYKGG	(SEQ ID NO: 7);
	GGTYSCHFGPLTWVCKPQGG	(SEQ ID NO: 8);
	GGDYHCRMGPPLTWVCKPLGG	(SEQ ID NO: 9);
	VGNYMCHFGPITWVCRPGGG	(SEQ ID NO: 10);
25	GGVYACRMGPITWVCSPLGG	(SEQ ID NO: 11);
	VGNYMAHMGPIWVCRPGG	(SEQ ID NO: 12);
	GGTYSCHFGPLTWVCKPQ	(SEQ ID NO: 13);
	GGLYACHMGPMPTWVCQPLRG	(SEQ ID NO: 14);
	TIAQYICYMGPETWECRPSPKA	(SEQ ID NO: 15);
30	YSCHFGPLTWVCK	(SEQ ID NO: 16);
	YCHFGPLTWVC	(SEQ ID NO: 17); and

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SCHFGPLTWVCK

(SEQ ID NO: 18).

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Other particularly preferred monomeric peptide units of the present dimers include peptides comprising a core sequence of the formula  $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 19) wherein  $X_2$  through  $X_8$  are as previously defined herein (SEQ ID NO: 2),  $n$  is 1 or 0 and  $A$  is any one of the naturally occurring L-amino acids except  $Y$  (tyrosine);  $n$  is defined herein as the number of occurrences of  $(AX_2)$  which can be 1 or none in the core sequence. When  $(AX_2)$  is present, i.e. when  $n = 1$ ,  $A$  is not tyrosine and  $A$  is not any non-naturally occurring aromatic amino acid analog. Such monomeric peptide units of the dimers of this invention can be prepared by truncating the peptides of Fig. 9, for example, from the N-terminus to delete the  $Y$ , tyrosine residue in SEQ ID NOS. 21 - 93. Such monomeric peptides can also be prepared by substitution of  $Y$  in position  $A$  in the peptides of Fig. 9.

In accordance with the present invention the monomeric units of the dimers can be the same or different.

In a preferred embodiment polyethylene glycol (PEG) is employed as a linker to form the dimeric peptides of the present invention through a covalent bond.

In another embodiment, the present invention is directed to pharmaceutical compositions comprising at least one dimer peptide of the invention and a pharmaceutical carrier.

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1 In a further embodiment, the present invention  
provides a method for therapeutically treating a mammal  
having a condition resulting from a hormone or growth  
5 factor deficiency by administration of at least one of  
the dimer peptides of the present invention.

10 In a still further embodiment, a method for  
therapeutically treating a mammal having a condition  
resulting from a deficiency of EPO or from reduced  
levels of blood oxygen caused by a decrease in  
erythrocyte number is provided.

15 In another embodiment of this invention, a  
method is provided for preparing agonists of cell-  
surface receptors wherein agonists of the class of cell-  
surface or dimerization-mediated receptors are dimerized  
to enhance the in vitro or in vivo biological activity  
of the cell-surface receptor relative to the monomeric  
agonists from which the dimer is derived. This method is  
also directed to the preparation of agonists of such  
20 growth-factor-type receptors by dimerizing antagonists  
of these receptors; the dimerized 'antagonists' thereby  
exhibit agonist biological activity in vitro and in  
vivo. In a preferred embodiment, the present method is  
directed to the preparation of EPO-R dimer agonists from  
monomeric EPO-R antagonists.

#### 25 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a major peak, with a retention  
time of 37 minutes, of the dimerized EPO peptide,  
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8)

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1                    Fig. 2 shows a major peak, with a retention  
time of 48 minutes, following purification of the  
dimerized EPO peptide, (SEQ ID NO: 8).

5                    Fig. 3 depicts the MALDI-TOF mass spectral  
analysis of the dimerized peptides, including peptide  
(SEQ ID NO: 8), GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13) and  
SCHFGPLTWVCK (SEQ ID NO: 18).

10                   Fig. 4 shows the SDS-PAGE analysis of DPDPB  
crosslinking of EPO binding protein (EBP) in the  
presence and absence of EPO agonist peptides.

15                   Fig. 5 demonstrates equilibrium EPO binding to  
immobilized EPO binding protein. Panel A represents the  
equilibrium binding data and Panel B (inset) is the  
linear transformation (Scatchard) of the data set in  
Panel A.

20                   Fig. 6 depicts the results of a competitive  
binding assay run on the EPO agonist peptide  
(SEQ ID NO: 8) in competitive binding with [ $^{125}$ I]EPO to  
EBP beads (Panel A); and EPO responsive cell  
proliferation studies in FDC-P1 derived cell lines  
containing either a human (Panel B) or murine EPO  
receptor (Panel C).

25                   Fig. 7 is a graphic representation of the  
results of the exhypoxic mouse bioassay; stimulation of  
the incorporation of [ $^{59}$ Fe] into nascent red blood cells  
by EPO, peptide (SEQ ID NO: 8) (Panel A) and peptide  
(SEQ ID NO: 8) dimer (Panel B).

30                   Fig. 8 demonstrates the effect of PEG  
dimerization of peptide (SEQ ID NO: 18) activity in EPO  
responsive cell proliferation studies in FDC-P1 derived  
cell lines containing a human EPO receptor.

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1           Fig. 9 provides the sequences of  
representative monomeric peptides of the present  
invention.

5       DETAILED DESCRIPTION OF THE INVENTION

10           The present invention is directed to peptide  
dimers which behave as cell surface receptor agonists,  
dimers which exhibit binding and signal initiation of  
growth-factor-type receptors. Sometimes called cell-  
surface receptors, growth-factor-type receptors or  
15       dimerization-mediated activator-receptors, these are a  
class of molecules which are understood to be activated  
by ligand-induced or ligand stabilized dimerization.  
Agonists of such receptors typically include large  
polypeptide hormones including the cytokines, insulin  
20       and various other growth or differentiation factors.  
The agonists are understood to induce dimerization of  
the receptor and thereby effect signal initiation. Such  
agonists are believed to effectively cross-link two  
receptors resulting in the repositioning of cytoplasmic  
25       tails which may directly or indirectly effect  
phosphorylation of the cytoplasmic tails and activation  
of a signaling pathway.

30           The present invention specifically includes  
those molecules which behave as agonists of cell-surface  
receptors when dimerized in accordance with this  
invention. Such dimer agonists can include 'monomeric'  
units which exhibit agonist or antagonist activity for  
the related receptor molecule and may be the same or  
different. The dimers are preferably peptides but may  
35       alternatively be small molecule pharmacophores. These  
molecules when dimerized exhibit agonist activity of

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1 cell-surface receptors in vitro and in vivo. Such  
receptors include, for example, EPO, GM-CSF, G-CSF, M-  
CSF, GH, EGF, PDGF, VEGF, Insulin and FGF. Other  
5 receptors which are activated by heterodimerization or  
multimerization may also be subject to activation by  
this mechanism including, IL-3, IL-5, IL-6, IL-2 and  
TPO. The dimers of the present invention have two  
'monomeric' peptide units of 10 to 40 or more amino  
10 acids, preferably 14 to about 20 amino acid residues in  
length. In a preferred embodiment, these monomeric  
peptide units comprise a core sequence of amino acids  
 $X_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 1) where each amino acid is  
indicated by standard one letter abbreviation;  $X_3$  can be  
15 C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid, or Hoc, where Hoc is  
homocysteine;  $X_4$  can be R, H, L, or W;  $X_5$  can be M, F,  
or I;  $X_6$  is independently selected from any one of the  
20 genetically coded L-amino acids or the stereoisomeric  
D-amino acids;  $X_7$  can be D, E, I, L, or V; and  $X_8$  can be  
C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid, or Hoc, where Hoc is  
homocysteine, provided that either  $X_3$  or  $X_8$  is C or Hoc.  
Preferably, the monomeric peptide unit of the dimer  
comprises a core sequence  $YX_2X_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID  
25 NO: 2) where each amino acid is indicated by standard  
one letter abbreviation; each  $X_2$  and  $X_6$  is independently  
selected from any one of the 20 genetically coded L-  
amino acids;  $X_3$  can be C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric  
acid, or Hoc, where Hoc is homocysteine;  $X_4$  can be R, H,  
L, or W;  $X_5$  can be M, F, or I;  $X_7$  can be D, E, I, L, or  
V; and  $X_8$  can be C, A,  $\alpha$ -amino- $\gamma$ -bromobutyric acid, or  
30 Hoc, where Hoc is homocysteine, provided that either  $X_3$   
or  $X_8$  is C or Hoc.

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1 More preferably, the monomeric peptide unit of  
the dimer comprises a core sequence of amino acids  
X<sub>1</sub>YX<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>X<sub>8</sub>X<sub>9</sub>X<sub>10</sub>X<sub>11</sub> (SEQ ID NO: 3), where each  
5 amino acid is indicated by standard one letter  
abbreviation; each X<sub>1</sub>, X<sub>2</sub>, X<sub>6</sub>, X<sub>9</sub>, X<sub>10</sub>, and X<sub>11</sub> is  
independently selected from any one of the 20  
genetically coded L-amino acids; X<sub>3</sub> can be C, A,  
α-amino-γ-bromobutyric acid, or Hoc, where Hoc is  
10 homocysteine; X<sub>4</sub> can be R, H, L, or W; X<sub>5</sub> can be M, F,  
or I; X<sub>7</sub> can be D, E, I, L or V; and X<sub>8</sub> can be C, A,  
α-amino-γ-bromobutyric acid, or Hoc, where Hoc is  
homocysteine, provided that either X<sub>3</sub> or X<sub>8</sub> is C or Hoc.

In a more preferred embodiment, both X<sub>3</sub> and X<sub>8</sub>  
15 will be C and thus, the monomeric peptide unit of the  
dimer comprises a core sequence of amino acids  
X<sub>1</sub>YX<sub>2</sub>CX<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>CX<sub>9</sub>X<sub>10</sub>X<sub>11</sub> (SEQ ID NO: 4). More  
preferably, the monomeric peptide unit comprises a core  
sequence of amino acids X<sub>1</sub>YX<sub>2</sub>CX<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>CX<sub>9</sub>X<sub>10</sub>X<sub>11</sub> (SEQ  
20 ID NO: 5), where X<sub>4</sub> can be R or H; X<sub>5</sub> can be F or M; X<sub>8</sub>  
can be I, L, T, M, or V; X<sub>7</sub> is D or V; X<sub>9</sub> can be G, K,  
L, Q, R, S, or T; and X<sub>10</sub> can be A, G, P, R, or Y. In a  
most preferred embodiment, the monomeric peptide unit of  
the dimer comprises a core sequence of amino acids  
25 X<sub>1</sub>YX<sub>2</sub>CX<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>CX<sub>9</sub>X<sub>10</sub>X<sub>11</sub> (SEQ ID NO: 6), where X<sub>1</sub> can  
be D, E, L, N, S, T, or V; X<sub>2</sub> can be A, H, K, L, M, S,  
or T; X<sub>4</sub> is R or H; X<sub>9</sub> can be K, R, S, or T; and X<sub>10</sub> is  
P. Particularly preferred monomeric peptide units of  
the present dimers include:

30 GGLYLCRFGPVTWDCGYKGG (SEQ ID NO: 7);

GGTYSCHFGPLTWCKPQGG (SEQ ID NO: 8);

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1 GGDYHCRMGPLTWVCKPLGG (SEQ ID NO: 9);  
VGNYMCHFGPITWVCRPGGG (SEQ ID NO: 10);  
GGVYACRMGPITWVCSPLGG (SEQ ID NO: 11);  
5 VGNMAHMGPIWVCRPGG (SEQ ID NO: 12);  
GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13);  
GGLYACHMGPMWVCPQPLRG (SEQ ID NO: 14);  
TIAQYICYMGPIWVCRPSPKA (SEQ ID NO: 15);  
YSCHFGPLTWVCK (SEQ ID NO: 16);  
10 YCHFGPLTWVC (SEQ ID NO: 17); and  
SCHFGPLTWVCK (SEQ ID NO: 18).

The dimer peptides of the present invention exhibit increased biological potency in vitro and in vivo relative to the monomeric agonists from which the dimers are derived. Moreover, cell surface receptor antagonists can be 'converted' to cell surface receptor agonists in accordance with the present invention. Specifically, a cell surface receptor antagonist can be dimerized with PEG or another appropriate linker which permits mutual binding of the monomeric moieties with the receptors. As a result, the dimer exhibits effective binding to the target receptor and behaves as an agonist. Accordingly, the dimers of this invention demonstrate enhanced biological potency in vitro and in vivo relative to their monomeric forms.

The dimer peptides of the present invention bind to and biologically activate the cell surface receptor or otherwise behave as agonists and are preferably formed by employing polyethylene glycol as a linker between the monomeric peptide units described herein. While other conventional chemical systems can

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1 also be employed to form the dimer peptides of this  
invention including using other known polymeric  
compounds, pegylation is preferred.

5 The linking compounds of the present invention  
include any molecule which covalently binds the  
monomeric peptides at an appropriate distance or which  
otherwise effects dimerization of the particular cell  
surface receptor thereby initiating biological efficacy.

10 Starting with an appropriate synthetic  
peptide, containing a free amino group or other reactive  
site such as hydroxyls, carboxylic acids or sulfhydryls,  
the peptide is added in excess to a reaction mixture  
containing a corresponding reactive polymer. The  
15 polymer can be of a repeating nature such as  
polyethylene glycol, peptides, modified peptides or  
peptide analogs. Alternatively, the peptide can be  
dimerized on a small molecule scaffold such as activated  
benodiazepins, oxazolones, azalactones, aminimides or  
20 diketopiperazines. The most readily available linker of  
variable distance are ones based on linear unbranched  
polyethylene glycols.

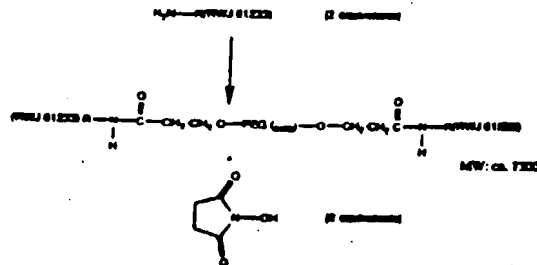
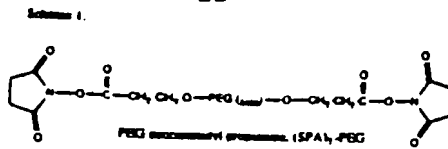
25 The following is a schematic of a preferred  
preparatory methodology employing PEG succinimidyl  
propionate as the linker between the monomer units of  
the dimer peptides.

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Dimerization and especially pegylation in a head-to-head (amino to amino terminus) or head-to-tail (amino to carboxyl terminus) configuration is preferred relative to internal covalent binding of the monomeric peptides. The 'monomer' units of the dimer peptides of the present invention can be the same or different, although the same are preferred.

The monomeric peptides which are used to form the dimers of the present invention can be prepared by classical chemical methods well known in the art. The standard methods include, for example, exclusive solid phase synthesis and recombinant DNA technology. See, e.g. Merrifield (1963) J. Am. Chem. Soc. **85**:2149. Solid phase synthesis is typically commenced from the C-terminal end of the peptide using an  $\alpha$ -amino protected resin. A suitable starting material can be prepared by attaching the required  $\alpha$ -amino acid to a chloromethylated resin (such as BIO-BEADS SX-1, Bio Rad Laboratories, Richmond, CA), a hydroxymethyl resin, (described by Bodonszky et al. (1966) Chem. Ind.

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1 (London) 38:1597) or a benzhydrylamine resin (described  
by Pietta and Marshall (1970) Chem. Commn. 650).

5 The  $\alpha$ -amino protecting groups are those known  
to be useful in the art of stepwise synthesis or  
peptides. Included are acyl type protecting groups  
(e.g. formyl, trifluoroacetyl, acetyl), aromatic  
urethane type protecting groups (e.g. benzyloxycarbonyl  
10 (Cbz) and substituted Cbz), aliphatic urethane  
protecting groups (e.g., t-butyloxycarbonyl (Boc),  
isopropylloxycarbonyl, cyclohexyloxycarbonyl) and alkyl  
type protecting groups (e.g., benzyl and  
triphenylmethyl). The preferred X-amino protecting  
group is Fmoc. The side chain protecting group  
15 (typically ethers, esters, trityl, PMC, and the like)  
remains intact during coupling and is not split off  
during the deprotection of the amino-terminus protecting  
group or during coupling. The side chain protecting  
group must be removable upon the completion of the  
20 synthesis of the final peptide and under reaction  
conditions that will not alter the target peptide.

The side chain protecting groups for Tyr  
include tetrahydropyranyl, tert-butyl, trityl, benzyl,  
Cbz, Z-Br-Cbz, and 2,5-dichlorobenzyl. The side chain  
protecting groups for Asp include benzyl, 2,6-  
25 dichlorobenzyl, methyl, ethyl, and cyclohexyl. The side  
chain protecting groups for Thr and Ser include acetyl,  
benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-  
dichlorobenzyl, and Cbz. The side chain protecting  
groups for Thr and Ser are benzyl. The side chain  
30 protecting groups for Arg include nitro, Tosyl. (Tcs),  
Cbz, adamantyloxycarbonyl mesitoysulfonyl (Mts), or

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1. Boc. The side chain protecting groups for Lys include  
Cbz, 2-chlorobenzyloxycarbonyl (2-Cl-Cbz), 2-  
bromobenzyloxycarbonyl (2-BrCbz), Tos, or Boc.

5 After removal of the  $\alpha$ -amino protecting group,  
the remaining protected amino acids are coupled stepwise  
in the desired order. Each protected amino acid is  
generally reacted in about a 3-fold excess using an  
appropriate carboxyl group activator such as 2-(1H-  
10 benxotriazol-1-yl)-1,1,3,3-tetramethyluronium  
hexafluorophosphate (HBTU) or dicyclohexylcarbodiimide  
(DCC) in solution of methylene chloride ( $\text{CH}_2\text{Cl}_2$ ), or  
dimethyl formamide (DMF) mixtures.

15 After the desired amino acid sequence has been  
completed, the desired peptide is decoupled from the  
resin support by treating the mixture with a reagent  
such as trifluoroacetic acid (TFA) or hydrogen fluoride  
(HF). These reagents not only cleave the peptide from  
the resin, but also cleave all remaining side chain  
20 protecting groups. When the chloromethylated resin is  
used, hydrogen fluoride treatment results in the  
formation of the free peptide acids. When the  
benzhydrylamine resin is used, hydrogen fluoride  
treatment results directly in the free peptide amide.  
25 Alternatively, when the chloromethylated resin is  
employed, the side chain protected peptide can be  
decoupled by treatment of the peptide resin with ammonia  
to give the desired side chain protected amide or with  
an alkylamine to give a side chain protected alkylamide  
or dialkylamide. Side chain protection is then removed  
30 in the usual fashion by treatment with hydrogen fluoride  
to give the free amides, alkylamides, or dialkylamides.

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1. These procedures can also be used to  
synthesize peptides in which amino acids other than the  
20 naturally occurring, genetically encoded amino acids  
are substituted at one, two or more positions of any of  
5 the compounds of the invention. For instance,  
naphthylalanine can be substituted for tryptophan,  
facilitating synthesis. Other synthetic amino acids  
that can be substituted into the peptides of the present  
invention include L-hydroxypropyl, L-3, 4-  
10 dehydroxyphenylalanyl,  $\delta$  amino acids such as L- $\delta$ -  
hydroxylysyl and D- $\delta$ -methylalanyl, L- $\alpha$ -methylalanyl,  $\beta$   
amino acids, and isoquinolyl. D-amino acids and non-  
naturally occurring synthetic amino acids can also be  
incorporated into the peptides of the present invention.

15 In another embodiment of the present  
invention, a method of enhancing the in vitro or in vivo  
biological potency of a cell surface receptor agonist is  
provided. This methodology is achieved by dimerizing  
the receptor agonist with a linker molecule, such as  
20 PEG, to form an appropriate spatial relationship between  
the monomeric peptide units of the dimer and thereby  
permitting each of the constituents of the dimers to  
bind to their receptors to achieve enhanced biological  
potency, i.e., to dimerize and thereby activate the  
25 receptors to induce the relevant biological activity of  
the particular cell-surface receptor, e.g. EPO-R.  
Biological activity can be measured by the skilled  
artisan in various in vitro and in vivo assays and as  
demonstrated in the examples of the present invention.

30 The peptide or molecule with binding affinity  
for a given receptor will have increased conformational

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1 flexibility leading to fewer barriers to effective  
receptor interaction and subsequently receptor  
activation. This is also indicated for molecules which  
5 can bind but not activate a receptor subtype in that  
such molecules can become more effective inhibitors of  
ligand binding.

The present invention further provides a  
method for altering a cell-surface receptor antagonist,  
a molecule exhibiting receptor binding but no biological  
10 activity, to behave as a cell-surface receptor agonist  
in vitro or in vivo. This method is achieved by  
dimerizing the antagonist molecule with an appropriate  
linker molecule such as PEG, other polymerized molecules  
or a peptide. In a preferred embodiment, an EPO  
15 antagonist, i.e. a peptide exhibiting receptor binding  
but no biological EPO activity can be altered by  
dimerization to obtain a dimer which behaves as an EPO  
receptor agonist. Thus, for example, in the case of  
EPO-R these include the monomeric peptide units of the  
20 present dimers comprising a core sequence of general  
formula  $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 19) wherein  
 $X_2$  through  $X_8$  are as previously defined herein, in (SEQ  
ID NO: 2),  $n$  is 1 or 0 and  $A$  is any one of the naturally  
occurring L-amino acids except Y (tyrosine);  $n$  is  
25 defined herein as the number of occurrences of  $(AX_2)$   
which can be 1 or none in the core sequence. When  $X_2$  is  
present, i.e., when  $n = 1$ ,  $A$  is not tyrosine and  $A$  is  
not any non-naturally occurring aromatic amino acid  
analog. Such monomeric peptide units of the dimers of  
30 this invention can be prepared by truncating the  
peptides of Fig. 9, for example, from the N-terminus to

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1 delete the Y, tyrosine residue in SEQ ID NOS. 21 - 93.  
Such monomeric peptides can also be prepared by  
substitution of Y in the peptides of Fig. 9.

5 These molecules, demonstrate only binding  
activity in their 'monomeric' form, but exhibit agonist  
activity after dimerization with a linking compound such  
as PEG. Accordingly, the present method comprises  
10 identifying a monomeric peptide as herein defined which  
does not demonstrate biological activity and dimerizing  
that antagonist in accordance with the present invention  
to obtain a cell-surface-receptor agonist i.e., in  
dimeric form. Contacting the appropriate cell-surface  
receptor with the thus formed dimer activates, i.e.  
15 dimerizes such receptors and thus induces biological  
activity of the receptor. Such monomeric units as  
shown in Fig. 9 can be truncated from the N-terminus  
such as SCHFGPLTWVCK (SEQ ID NO: 18) to eliminate the  
tyrosine residue at position A of the formula  
20  $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 19) or merely  
substituted with any of the remaining 19 naturally  
occurring amino acids or with other than a non-naturally  
occurring aromatic amino acid analog. In accordance  
with the present invention it has been determined that  
25 the tyrosine residue at position A of the foregoing  
formula is critical to biological activity of the  
monomer peptide. Deletion or substitution of the  
tyrosine eliminates biological activity. When dimerized  
however the entity exhibits enhanced biological  
activity.

30 For example, tyrosine (Y) substituted in the  
formula  $YX_2X_3X_4X_5GPX_7X_8$  (SEQ ID NO: 2) by p-

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1       iodohydroxyphenylalanine, p-fluorohydroxyphenylalanine,  
p-amino-hydroxyphenylalanine act as EPO-R monomer  
agonists but substitution with threonine or alanine for  
5       tyrosine at position Y causes the monomer peptide to act  
as an EPO-R antagonist. However, when dimerized in  
accordance with the present invention, such dimers  
behave as EPO-R agonists. The monomeric peptide units  
identified at Fig. 9, for example, behave as EPO-R  
10       antagonists in the absence of tyrosine at position Y of  
the formula above. When such antagonists are dimerized,  
the dimer behaves as an EPO-R agonist.

In a further embodiment of the present  
invention, pharmaceutical compositions comprising at  
least one of the dimers of this invention can be  
15       employed to therapeutically treat disorders resulting  
from deficiencies of biological factors such as EPO, GH,  
GM-CSF, G-CSF, EGF, PDGF, VEGF, insulin, FGF and the  
like. These pharmaceutical compositions may contain  
buffers, salts and other excipients to stabilize the  
20       composition or assist in the delivery of the dimerized  
molecules.

In a preferred embodiment, the present  
invention provides a method for treating disorders  
associated with a deficiency of EPO. The method is  
25       accomplished by administering at least one of the dimers  
identified herein for a time and under conditions  
sufficient to alleviate the symptoms of the disorder,  
i.e. sufficient to effect dimerization or biological  
activation of EPO receptors. In the case of EPO such  
30       methodology is useful in the treatment of end-stage  
renal failure/dialysis; anemia, especially associated

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1 with AIDS or chronic inflammatory diseases such as  
rheumatoid arthritis and chronic bowel inflammation;  
auto-immune disease; and for boosting the red blood cell  
5 count of patient when necessary, e.g. prior to surgery  
or as pretreatment to transfusion. The dimers of the  
present invention which behave as EPO agonists can be  
used to activate megakaryocytes.

10 Since EPO has been shown to have a mitogenic  
and chemotactic effect on vascular endothelial cells as  
well as an effect on central cholinergic neurons (see,  
e.g., Amagnostou et al. (1990) Proc. Natl. Acad. Sci.  
USA 87:597805982 and Konishi et al. (1993) Brain Res.  
15 609:29-35), the compounds of this invention can also be  
used to treat a variety of vascular disorders, such as  
promoting wound healing, growth of collateral coronary  
blood vessels (such as those that may occur after  
myocardial infarction), trauma, and post vascular graft  
treatment, and a variety of neurological disorders,  
20 generally characterized by low absolute levels of acetyl  
choline or low relative levels of acetyl choline as  
compared to other neuroactive substances e.g.,  
neurotransmitters.

25 Accordingly, the present invention includes  
pharmaceutical compositions comprising, as an active  
ingredient, at least one of the peptide dimers of the  
present invention in association with a pharmaceutical  
carrier or diluent. The dimers of this invention can be  
administered by oral, parenteral (intramuscular,  
30 intraperitoneal, intravenous (IV) or subcutaneous  
injection), transdermal (either passively or using  
iontophoresis or electroporation) or transmucosal

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1 (nasal, vaginal, rectal, or sublingual) routes of  
administration in dosage forms appropriate for each  
route of administration.

5 Solid dosage forms for oral administration  
include capsules, tablets, pill, powders, and granules.  
In such solid dosage forms, the active compound is  
admixed with at least one inert pharmaceutically  
acceptable carrier such as sucrose, lactose, or starch.  
10 Such dosage forms can also comprise, as it normal  
practice, additional substances other than inert  
diluents, e.g., lubricating, agents such as magnesium  
stearate. In the case of capsules, tablets and pills,  
the dosage forms may also comprise buffering, agents.  
15 Tablets and pills can additionally be prepared with  
enteric coatings.

Liquid dosage forms for oral administration  
include pharmaceutically acceptable emulsions,  
solutions, suspensions, syrups, with the elixirs  
20 containing inert diluents commonly used in the art, such  
as water. Besides such inert diluents, compositions can  
also include adjuvants, such as wetting agents,  
emulsifying and suspending agents, and sweetening,  
flavoring and perfuming agents.

25 Preparations according to this invention for  
parenteral administration include sterile aqueous or  
non-aqueous solutions, suspensions, or emulsions.  
Examples of non-aqueous solvents or vehicles are  
propylene glycol, polyethylene glycol, vegetable oils,  
30 such as olive oil and corn oil, gelatin, and injectable  
organic esters such as ethyl oleate. Such dosage forms  
may also contain adjuvants such as preserving, wetting,

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1 emulsifying, and dispersing agents. They may be  
sterilized by, for example, filtration through a  
bacteria retaining filter, by incorporating sterilizing  
5 agents into the compositions, by irradiating the  
compositions, or by heating the compositions. They can  
also be manufactured using sterile water, or some other  
sterile injectable medium, immediately before use.

Compositions for rectal or vaginal  
10 administration are preferably suppositories which may  
contain, in addition to the active substance, excipients  
such as cocoa butter or a suppository wax. Compositions  
for nasal or sublingual administration are also prepared  
with standard excipients well known in the art.

15 The dosage of active ingredient in the  
compositions of this invention may be varied; however,  
it is necessary that the amount of the active ingredient  
shall be such that a suitable dosage form is obtained.  
The selected dosage depends upon the desired therapeutic  
20 effect, on the route of administration, and on the  
duration of the treatment desired. Generally dosage  
levels of between 0.001 to 10 mg/kg of body weight daily  
are administered to mammals.

As can be appreciated from the disclosure  
above, the present invention has a wide variety of  
25 applications. Accordingly, the following examples are  
offered by way of illustration, not by way of  
limitation.

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EXAMPLE 1

SDS-PAGE gels (10-20% gradient SDS-PAGE plates, 84 x 70 x 1.0 mm, Integrated Separation Systems, Natick, MA) were stained with Coomassie Brilliant Blue R-250 (BioRad). A commercial preparation of activated difunctional polyethylene glycol (PEG-succinimidyl-propionate, SPA2, MW ca. 3400) was purchased from Shearwater Polymers, Huntsville, AL as was the monofunctional reagent, methoxy-PEG-succinimidyl-propionate, MW ca 5000. Peptide (SEQ ID NO: 8) and all other peptides were obtained from the Peptide Synthesis Facility RWJ-PRI, La Jolla, CA or Quality Controlled Biochemical, Hopkinton MA. These peptides were cyclized via oxidation of their intramolecular cysteines, amidated at the C-terminus and mass confirmed by FAB-MS. All were Ellman Reaction negative. Tris base was obtained from BioRad, Hercules, CA. (DPDPB) and trifluoroacetic acid (HPLC grade) were obtained from Pierce Chemical Co., Rockford IL.

Mono-PEG conjugation of peptide GGTYSCHFGPLTWCKPQGG  
(SEQ ID NO: 8)

This example describes the preparation of mono-PEG conjugates of peptide (SEQ ID NO: 8), using the monofunctional amine reactive polymer analog m-SPA-PEG to be used as a control in experiments described herein. The reaction was carried out with polymer in excess (ca. 3 fold) by resuspending 142.5 mg (0.0286 mmol, MW ca. 5000) of polymer in 4 ml PBS at pH 7.5 and adding 20 mg peptide (SEQ ID NO: 8) (0.0095 mmol, MW 2092) dissolved

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1 in 1 ml of 0.1% trifluoroacetic acid. The mixture was  
incubated on ice for 20 hours. The reaction was  
subsequently adjusted to a final concentration 50 mM  
5 Tris by the addition of 1 M tris-HCl at pH 7.5. The  
reaction mixture was incubated on ice for one hour.  
Analytical HPLC suggested that there were two main  
reaction products of essentially equivalent magnitude  
which were not baseline resolved. Preparative HPLC  
10 (using the flatter gradient system described in Example  
8) and conservative cuts resulted in collection of two  
product peaks eluting at ca 44 and 47 minutes. After  
lyophilization, 24.8 mg, 16.5 mg of each species was  
recovered, respectively. Mass spectral analysis of  
15 these two species demonstrated centroid masses of 7092  
(peak 1) and 12036 (peak 2) indicating the coupling of  
one or two PEG molecules, respectively, to the peptide  
(Table I).

Tris inactivated polymer. Tris inactivated  
polymer was formed by incubation of 5 mM SPA2 polymer  
20 dissolved in PBS (Gibco, Gaithersburg, MD) with 50 mM  
tris-HCl, pH 7.5 added and used without further  
purification.

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TABLE 1  
Recovery Yield of Peptide Conjugation Reaction and Apparent Molecular Mass of Product

I.D. No.	Sequence	Mass	Conjugation Reagent	Main Product Mass (centroid m/z)	Yield (% of theoretical)
8	GGTYSCHFGLTWCKPQG	2092	SPA2-PEG (MW ca. 3400)	7834	69
13	GGTYSCHFGLTWCKPQ	1978	m-SPA-PEG (MW ca. 5000)	7092 (peak 1) 12036 (peak 2)	-
20	Ac-GGTYSCHFGLTWCKPQG	2133	SPA2-PEG	7560	54
14	GGLYACHMGPHTWVCPLRG	2177	SPA2-PEG	7862	30
18	SCHFGLTWCK	1375	SPA2-PEG	7872	37
				6326	45

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EXAMPLE 2

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PEG dimerization of peptide GGTYSCHFGPLTWVCKPQGG  
(SEQ ID NO: 8) (lot #1)

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Examples 2 - 7 describe the dimerization of various peptides described by the present invention. The modification of peptide (SEQ ID NO: 8) was carried out by resuspending 25 mg (0.0071 mmol) of polymer in 4 ml PBS at pH 7.5, and adding a 3 fold molar excess of peptide (SEQ ID NO: 8) (0.0213 mmol, 44.5 mg, MW 2092) dissolved in 1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice. After 3 hours of incubation, an additional 7.5 mg (0.0036 mmol) of lyophilized peptide was added, resulting in a final ratio of 3.5 moles of peptide per each mole of SPA2. The mixture was incubated an additional 17 hours on ice. The reaction mixture was adjusted to a final concentration of 50 mM Tris by the addition of 1 M tris-HCl of pH 7.5 and incubated on ice for 1 hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. After preparative HPLC and lyophilization, 38 mg of PEG dimer was recovered. The theoretical yield for this experiment was 55 mg based on a calculated mass of 7600 mg/mmol for a yield of 69% (Table I).

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EXAMPLE 3

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PEG dimerization of peptide GGTYSCHFGPLTWVCKPQGG  
(SEQ ID NO: 8) (lot #2)

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The modification of peptide (SEQ ID NO: 8) was carried out by resuspending 25 mg (0.0071 mmol) of polymer in 4 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 8) (0.0213 mmol, 45.8 mg, MW 2092) dissolved in 1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 22 hours. At that time, the reaction was adjusted to a final concentration of 50 mM Tris by the addition of 1 M tris-HCl, pH 7.5. The reaction mixture was incubated on ice for 1 hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. After preparative HPLC and lyophilization, 37 mg of PEG dimer was recovered. The theoretical yield for this experiment was 55 mg based on a calculated mass of 7600 mg/mmol for a yield of 68% (Table I).

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EXAMPLE 4

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PEG dimerization of peptide GGTYSCHFGPLTWVCKPQ  
(SEQ ID NO: 13)

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The modification of peptide (SEQ ID NO: 13) was carried out by resuspending 11.2 mg (0.0033 mmol) of polymer in 2.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 13) (0.010 mmol, 20 mg, MW 1978) dissolved in 0.25 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 20 hours. At that time, 0.25 ml of 1 M tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for one hour. The sample was subjected to analytical and preparative HPLC as described in Example 8. The main preparative reaction product peak eluted at ca 43 minutes. After preparative HPLC and lyophilization, 13.3 mg of PEG dimer was recovered. The theoretical yield for this experiment was 24.42 mg based on a calculated mass of 7400 mg/mmol for a yield of 54% (Table I).



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EXAMPLE 5PEG dimerization of peptide Ac-GGTYSCHFGPLTWVCKPOGG

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(SEQ ID NO: 20)

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The modification of peptide (SEQ ID NO: 20) was carried out by resuspending 10.5 mg (0.0031 mmol) of polymer in 2.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 20) (0.0094 mmol, 20 mg, MW 2133) dissolved in 0.25 ml of 0.1% trifluoroacetic acid and the mixture incubated at 4°C for 28 hours. At that time, the reaction as monitored by HPLC was estimated to be approximately 30% complete, the temperature was shifted to ambient and an additional 27 hour incubation provided no net increase in product. Because of possible hydrolysis of the reactive polymer, an additional 5 mg of polymer was added and the incubation was continued for an additional 16 hours. At that time, 0.25 ml of 1 M tris-HCl, pH 7.5 was added and the reaction mix was incubated at 4°C for an additional 1 hour. The sample was subjected to analytical and preparative HPLC using a flatter gradient system as described in Example 8. The main preparative reaction product peak eluting at ca 48 minutes. After preparative HPLC and lyophilization, 10.4 mg of PEG dimer was recovered. The theoretical yield for this experiment was 34.4 mg based on a calculated mass of 7650 mg/mmol for a yield of 30% (Table I).

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EXAMPLE 6PEG dimerization of peptide (SEQ ID NO: 14)

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The modification of peptide (SEQ ID NO: 14) was carried out by resuspending 2.6 mg (0.00076 mmol) of polymer in 3.0 ml PBS at pH 7.5 and adding a ca. 3 fold molar excess of peptide (SEQ ID NO: 14) (0.00229 mmol, 5 mg, MW 2177) dissolved in 0.1 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 26 hours. At that time, 0.25 ml of 1 M-tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for 1 hour. The sample was subjected to analytical and preparative HPLC using the flatter gradient system described in Example 8. The main preparative reaction product peak eluted at ca 46 minutes. After preparative HPLC and lyophilization, 2.2 mg of PEG dimer was recovered. The theoretical yield for this experiment was 24.42 mg based on a calculated mass of 7400 mg/mmol for a yield of 37% (Table I).

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EXAMPLE 7PEG dimerization of peptide (SEQ ID NO: 18)

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The modification of peptide (SEQ ID NO: 18) was carried out by resuspending 1.2 mg (0.00036 mmol) of polymer in 0.5 ml PBS at pH 7.5, and adding a ca. 3 fold molar excess of the peptide (0.0011 mmol, 1.5 mg, MW 2177) dissolved in 0.05 ml of 0.1% trifluoroacetic acid. The mixture was incubated on ice for 20 hours. At that time, 0.1 ml of 1 M tris-HCl at pH 7.5 was added. The reaction mixture was incubated at 4°C for 1 hour. The sample was subjected to purification using an analytical HPLC system as described in Example 8. The main reaction product peak eluted at ca 38 minutes. After preparative HPLC and lyophilization, 1 mg of PEG dimer was recovered. The theoretical yield for this experiment was 2.2 mg based on a calculated mass of 6150 mg/mmol for a yield of 45% (Table I).

EXAMPLE 8Analytical and Preparative HPLC analysis

The accumulation of the dimers described above in Examples 1-7 was monitored by analytical reverse phase HPLC. The analysis was carried out using a Vydac C-18 Protein-Peptide column (0.46 x 25 cm, part no. 218TP54) and a Rainin Gradient HPLC system fitted with a Dynamax dual wavelength detector. At injection, the column was equilibrated in 0.1% TFA in dH<sub>2</sub>O and was developed with a 45 minute linear gradient (0-100%) of acetonitrile (ACN) containing 0.1% TFA beginning at 10 minutes after injection. The flow rate was held constant at 1 ml/min. Under these analytical conditions, the SPA2 polymer and tris inactivated polymer did not appear to bind the column while a major reaction product with a retention time (37 minutes) was identified (Figure 1). Peptide (SEQ ID NO: 8) demonstrated a retention time of 35 minutes and the excess peptide utilized in the reaction was clearly distinguished from the nascent reaction products.

The main product reaction product peak was purified by preparative reverse phase HPLC on the same chromatographic system using a Vydac C-18 Protein-Peptide column (2.2 x 25 cm, part no. 218TP15022). Injection of the reaction mix (6 ml) occurred with the column equilibrated at 80:20, H<sub>2</sub>O:ACN (both containing 0.1% TFA) at a constant flow rate of 8 ml/min. After a 20 minute wash, the column was developed by application of a linear gradient of 100% ACN/0/1% TFA over 60

-35-

1 minutes. The major product peak eluting at 48 minutes  
was collected and lyophilized (Figure 2). These elution  
conditions were subsequently modified to improve the  
5 resolution of some conjugation products peptide (SEQ ID  
NO: 20), mPEG-peptide (SEQ ID NO: 8), peptide (SEQ ID  
NO: 14) from reaction by products. This was  
accomplished by application of a flatter linear gradient  
of 20-80% B over 60 minutes. The variation in retention  
10 time due to different peptides and elution condition is  
described as part of each synthesis example. The  
materials recovered from the main product peak from each  
reaction were subsequently analyzed by analytical  
reverse phase HPLC, MALDI-TOF mass spectrometry, EPO  
15 competitive binding potential and for in vitro  
bioactivity.

The activated PEG used in these experiments  
has an approximate molecular weight of 3400 and has  
amine reactive succinimidyl groups on either end of the  
difunctional linear polymer. This reactivity was  
20 employed to couple two equivalents of peptide (SEQ ID  
NO: 8) (MW= 2092) to the polymer with the concomitant  
liberation of two succinimidyl moieties resulting in a  
dimeric product as shown in Scheme I. Peptide (SEQ ID  
25 NO: 8) contains two potentially reactive amines, one at  
the N-terminus of the peptide and one in the side chain  
of the single lysine within the peptide sequence, so  
that a number of different connectivities between the  
two molecules was possible.

30 MALDI-TOF mass spectral analysis was  
supportive of the presence of the expected dimeric  
product (Figure 3) as indicated by a predominant species.

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1 with a centroid mass of 7661. This data shows that the  
dimeric product described in the present invention was  
5 produced using the methods described herein.

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EXAMPLE 9EBP (EPO Binding Protein) Dimerization

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This example demonstrates the interaction of peptide (SEQ ID NO: 8), peptide (SEQ ID NO: 16), peptide (SEQ ID NO: 18) and peptide (SEQ ID NO: 13) with EPO binding protein (EBP) using a bifunctional sulphydryl reactive crosslinker, (1,4-Di-[3'-(2'-pyridyldithio)propionamido]butane DPDPB.

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To explore the interaction of peptide (SEQ ID NO: 8) with EBP, a bifunctional sulphydryl reactive crosslinker (DPDPB) was used in an attempt to stabilize a mimetic dependent dimeric structure. Control experiments demonstrated that the crosslinker does not inactivate the EPO binding potential of EBP or the proliferative properties of peptide (SEQ ID NO: 8). As shown in Figure 4, a dimeric EBP product was formed by co-incubation of the peptide, peptide (SEQ ID NO: 8), DPDPB and EBP. This data shows the ability of the peptide (SEQ ID NO: 8) to mediate formation of a soluble receptor dimer. To further explore this question, peptides (SEQ ID NO: 13), (SEQ ID NO: 16) and (SEQ ID NO: 18) were examined for their ability to mediate dimerization. As shown in Figure 4, lanes 7A and 8A, when peptide (SEQ ID NO: 13) was truncated at the carboxyl terminus, it retained good in vitro bioactivity and improved in vivo bioactivity, resulting in a crosslinking signal similar to peptide (SEQ ID NO: 8). However, peptide (SEQ ID NO: 18) did not appear to stabilize the dimerization signal (Figure 4, lanes 9A

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1 and 10A) whereas peptide (SEQ ID NO: 16) (Figure 4,  
lanes 5A and 6A) gave a strong dimerization band. These  
two peptides differ by a single N-terminal tyrosine  
5 residue and display a similar profile in the in vitro  
proliferation assay with peptide (SEQ ID NO: 18) being  
inactive. Peptide (SEQ ID NO: 16) has an ED<sub>50</sub> of 3  $\mu$ M  
on murine receptor cells. Both peptides have similar  
IC<sub>50</sub> values indicating that they both retain binding  
10 activity. These results demonstrate that EBP  
dimerization is a property of the EPO peptide series and  
that the presence of the tyrosine is critical for this  
activity and that this corresponds to in vitro  
bioactivity.



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EXAMPLE 10

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IMMOBILIZED EBP BASED [ $^{125}$ I]EPO COMPETITION BINDING  
ASSAY

This study examined the binding capacity of the EPO PEG dimers to bind EPO receptors.

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The extracellular domain of the human erythropoietin receptor (EPO binding protein, EBP) was expressed and overproduced in E. coli. As with many other recombinant eukaryotic proteins produced in E. coli, the protein appeared as an insoluble product in laboratory scale fermentations and was refolded and purified to obtain active protein. EPO binding protein produced by this method contains one free sulfhydryl group which can be modified without effecting the solution phase binding of ligand. In order to immobilize the EPO binding protein for equilibrium binding analysis and for competition binding assay, the EPO binding protein was covalently attached to agarose beads.

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The iodoacetyl activation chemistry of Sulfolink beads (Pierce Chemical Co, Rockford, IL) is specific for free thiols and assures that the linkage is not easily reversible. EBP-Sulfolink beads were made as follows: SulfoLink gel suspension (10 ml) was mixed with of coupling buffer (40 ml: 50 mM Tris, pH 8.3, 5 mM EDTA) and the gel was allowed to settle. The supernatant was removed and the EPO binding protein (0.3-1 mg/ml in coupling buffer) to be bound was added directly to the washed beads. The mixture was rocked

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1 gently for 30 minutes at room temperature and the beads  
were allowed to settle for 1 hour at room temperature.  
The supernatant was removed and retained. The beads  
5 were washed twice with 20 ml of coupling buffer. The  
washes were recovered as well. The beads were then  
treated with 20 ml of 0.05 M cysteine for 30 minutes at  
room temperature to block unbound sites. Finally, the  
beads were washed with 50 ml of 1 M NaCl, then with 30  
10 ml of PBS, and resuspended in 20 ml of PBS and stored at  
4°C. The amount of EBP which was covalently bound to  
the beads was determined by comparing the OD<sub>280</sub> of the  
original EBP solution to the total OD<sub>280</sub> recovered in  
the reaction supernatant and the two 20 ml washes.  
Typically, 40-60% of the applied EBP remains associated  
15 with the beads.

Binding assays were initiated by the addition  
of EPO binding protein beads (50 µl) to individual  
reaction tubes. Total binding was measured in tubes  
containing 0.3-30 nM [<sup>125</sup>I]EPO (NEN Research Products,  
20 Boston MA, 100 µCi/µg). For determination of  
non-specific binding, unlabelled EPO was added at a  
level of 1000 fold in excess of the corresponding  
[<sup>125</sup>I]EPO concentration. Each reaction volume was  
brought to 500 µl with binding buffer (PBS/0.2% BSA).  
25 The tubes were incubated for five hours (a time period  
experimentally determined as adequate for the  
establishment of equilibrium) at room temperature with  
gentle rocking. After five hours, each reaction mixture  
was passed through a 1 ml pipet tip plugged with glass  
30 wool. The tubes were washed with 1 ml wash buffer (PBS/  
5% BSA) and this volume as well as 2 additional 1 ml

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1 washes were passed through the pipet tip and collected  
for determination of the free EPO concentration.  
Equilibrium binding analysis of the specific association  
of [ $^{125}$ I]EPO with EPO mimetic binding proteins  
5 immobilized on these agarose beads indicates a  $K_d$  of 5  
nM  $\pm$  2 based on a linear transformation (Scatchard) of  
the binding isotherm (Figure 5).

Competitive binding analysis assays of  
candidate peptides and dimer peptides were performed as  
10 outlined below. Individual peptides were dissolved in  
DMSO to prepare a stock solution 1 mM. Dimer peptides  
were contained within PBS at a concentration of 5 mM.  
All reaction tubes (in duplicate) contained 50  $\mu$ L of EBP  
beads, 0.5 nM [ $^{125}$ I]EPO and 0-500  $\mu$ M peptide in a total  
15 of 500  $\mu$ L binding buffer.

The final concentration of DMSO was adjusted  
to 2.5% in all peptide assay tubes. At this  
concentration DMSO has no detectable effect since an  
examination of the sensitivity of the assay to DMSO  
20 demonstrated that concentrations of up to 25% DMSO (V/V)  
had no deleterious effect on binding. Non-specific  
binding was measured in each individual assay by  
inclusion of tubes containing a large excess of  
unlabelled EPO (1000 nM). Initial assay points with no  
25 added peptide were included in each assay to determine  
total binding. Binding mixtures were incubated  
overnight at room temperature with gentle rocking. The  
beads were then collected using Micro-columns (Isolab,  
Inc.) and washed with 3 mL of wash buffer. The columns  
30 containing the washed beads were placed in 12 x 75 mm  
glass tubes and bound radioactivity levels determined in

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1 a gamma counter. The amount of bound [ $^{125}$ I]EPO was  
expressed as a percentage of the control (total=100%)  
binding and plotted versus the peptide concentration  
5 after correction for non-specific binding. The  $IC_{50}$  was  
defined as the concentration of the analyte which  
reduced the binding of [ $^{125}$ I]EPO to the EBP beads by  
50%. All data are reported as relative to peptide (SEQ  
ID NO: 8) which demonstrated an  $IC_{50}$  of 5  $\mu$ M.

10 Competitive binding analysis revealed an  $IC_{50}$   
of 20  $\mu$ M for the purified dimer, a value four fold  
greater than peptide (SEQ ID NO: 8) in the same assay  
(Figure 5 and Table II). Polymer alone, which was  
inactivated by treatment with Tris-HCl, demonstrated a  
15 detectable competition binding signal but this signal  
was modest (<10%) at the  $IC_{50}$  of the PEG-peptide (SEQ ID  
NO: 8) dimer.

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TABLE II

Table II. Binding and Cell Proliferation Studies

id	Relative Binding <sup>a</sup>	EPO-ED <sub>50</sub> (μM) <sup>b</sup>	
		murine receptor	transfected human receptor
D. No. 8	1	0.1	0.09
ris inact.polymer	60	1A <sup>c</sup>	1A
q. I.D. No. 8 covalent dimer #1	4	0.01 (10X)	0.0015 (60X)
q. I.D. No. 8 covalent dimer #2	3	0.01 (10X)	0.002 (45X)
D. No. 13	1.6	0.08	0.02
sq. I.D. No. 13 covalent dimer	3	0.01 (8X)	0.002 (10X)
D. No. 20 (N-acetyl)	4	0.03	0.06
sq. I.D. No. 20 covalent dimer	12	0.2 (-7X)	0.05
D. No. 14 (terminal NH <sub>2</sub> )	0.6	0.1	0.08
sq. I.D. No. 14 covalent dimer	-	0.006 (16X)	0.001 (80X)

<sup>a</sup> Amount required to achieve the half maximal level of EPO dependent proliferation (11pM)

<sup>b</sup> Binding relative to Seq. I.D. No. 8

<sup>c</sup> 1A inactive

<sup>d</sup> Note that all peptides are cyclic and were analyzed as COOH terminal amides (-CONH<sub>2</sub>)

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EXAMPLE 11EPO DEPENDENT CELL PROLIFERATION ASSAYS

This example shows the improved potency of PEG-EPO peptide dimers to EPO receptors in human and murine cell lines.

Cell line FDC-P1/ER, an EPO-dependent line expressing the murine EPO receptor, was grown and maintained as described previously (Carroll et al. 1991). Also employed was cell line FDC-P1/trER expressing a functional truncated human EPO receptor (missing the C-terminal 40 amino acids). Both cell lines exhibit EPO dependent cellular proliferation. Briefly, cells were maintained in RPMI 1640 media (Gibco/BRL) containing 10% heat-inactivated fetal calf serum and 10 units/ml of recombinant human EPO. For the cellular proliferation assay, FDC-P1/ER or FDC-P1/trER cells were grown to stationary phase, centrifuged, washed with RPMI 1640 media (no EPO), and plated in EPO minus media for 24 hr.

After 24 hours, the cells were counted, resuspended at 800,000 cells/ml and dispensed at 40,000 cells/well. Stock solutions of the peptide dimer (5 mM in PBS) and peptide (10 mM in DMSO) were prepared and dispensed in triplicate to final concentrations of  $1 \times 10^{-10}$  M through  $1 \times 10^{-5}$  M and adjusted to a final volume of 0.2 ml. Final DMSO concentrations of 0.1% (V/V, maximal) or less were found to have no cellular toxicity or stimulatory effects. A standard EPO dose response curve was generated with each assay series.

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1 After a 42 hr incubation at 37°C (ca. 2 cell doublings)  
1  $\mu$ Ci/well of [ $^3$ H] thymidine was added and the incubation  
continued for 6 hr at which time the cells were  
5 harvested and counted to assess [ $^3$ H]thymidine  
incorporation as a measure of cell proliferation.  
Results are expressed as the amount of peptide or dimer  
peptide necessary to yield one half of the maximal  
activity obtained with recombinant EPO.

10 As shown in Figure 5 and Table II, the initial  
lot of PEG-peptide (SEQ ID NO: 8) dimer demonstrated  
ED<sub>50</sub> values of 0.01  $\mu$ M and 0.0015  $\mu$ M in EPO responsive  
cell lines containing the murine or human EPO receptor,  
respectively. In both cell lines, the parent peptide,  
15 peptide (SEQ ID NO: 8), demonstrated an ED<sub>50</sub> of 0.1  $\mu$ M,  
indicating an increase in potency of 10 fold in the  
murine receptor line and almost 60 fold in the human  
receptor containing cells. Thus, the dimer was clearly  
more potent in murine and human lines than the peptides  
themselves. This was confirmed by generation of a  
20 second synthesis lot of PEG-peptide (SEQ ID NO: 8) dimer  
which resulted in a 10 and 45 fold increase in potency  
in the murine and human lines, respectively. Polymer  
alone, which was inactivated by treatment with Tris-HCl,  
demonstrated no activity in the cell proliferation  
25 assay.

30 A second EPO mimetic peptide, peptide (SEQ ID  
NO: 13), with the sequence GGTYSCHFGPLTWCKPQ, was also  
subjected to a similar PEG dimerization protocol as that  
described above for peptide (SEQ ID NO: 8). The dimer  
product of PEG-peptide (SEQ ID NO: 13) is also more  
active than the unconjugated parent compound (Table II).

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1 Both of these dimer peptides have ultimate ED<sub>50</sub> values  
near 0.002 $\mu$ M. In spite of this more modest increase,  
the experimental evidence clearly indicates that the  
5 dimerization of these peptides with PEG results in  
improved potency.

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EXAMPLE 12

To further examine the connectivity of the peptides of the present invention to PEG, peptide molecules, which contained only an internal lysine group were used peptide (SEQ ID NO: 8) analog acetylated at the N-terminus peptide (SEQ ID NO: 20) and a sequence analog peptide (SEQ ID NO: 14) which only had a reactive N-terminal amine were PEG dimerized. In vitro proliferation data of these compounds suggest that potential dimerization through the free amino terminus has the most profound effect on bioactivity giving rise to a species about 80 fold more active than the monomeric parent peptide (SEQ ID NO: 14) dimer. Conjugation through the lysine side chain had no real effect on activity peptide (SEQ ID NO: 20) as did mono-PEG or di-PEG conjugation (Table III). This data indicates that the creation of a head to head dimer (both peptides attached through the N-terminus) using a PEG linker greatly enhances the potency of EPO peptides and approaches a level almost two logs greater than the free parent peptide. Further, this effect was not observed upon simple covalent attachment of linear PEG to peptide (SEQ ID NO: 8) indicating that dimerization is a critical determinant for this increased activity.

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TABLE III

Table III. Binding and Cell Proliferation Studies mPEG/

Compound	Relative Binding <sup>a</sup>	EPO-ED <sub>50</sub> (μM) <sup>a</sup>	
		murine receptor	truncated human receptor
Seq. I.D. No. 8	1	0.1	0.09
mPEG/Seq. I.D. No. 8, peak #1	60	2	0.1
mPEG/Seq. I.D. No. 8, peak #2	>40	1	0.4

<sup>a</sup> Amount required to achieve the half maximal level of EPO dependent proliferation (11pM)

<sup>b</sup> ND=Not determined

<sup>c</sup> As inactive

<sup>d</sup> Binding relative to Seq. I.D. No. 8

Note that all peptides are cyclic and were analyzed as COOH terminal amides (-CONH<sub>2</sub>)

EXAMPLE 13Polycythemic Exhyposic Mouse Bioassay.

This study demonstrates the ability of peptide (SEQ ID NO: 8)/PEG-dimers to retain in vivo bioactivity. Peptides were assayed for in vivo activity in the polycythemic mouse bioassay adapted from the method described by Cotes and Bangham (1961), Nature 191: 1065-1067. BDF1 mice were allowed to acclimate to ambient conditions for 7-10 days. Body weights were determined for all animals. Low weight animals (<15 grams) were not used. Mice were introduced to hypobaric chambers with a 24 hour conditioning cycle consisting of 0.40% +/- 0.02 atm. for 18 hours followed by 6 hours at ambient pressure for a total of 14 days. Following the 14 day period, mice were placed in ambient pressure for 72 hours prior to dosing. Test samples or recombinant Human Erythropoietin (rHuEPO) standards were diluted in an assay vehicle consisting of Phosphate Buffered Saline (PBS)-0.1% Bovine Serum Albumin (BSA). Peptide sample stock solutions (excluding peptide dimers) were first solubilized in dimethyl sulfoxide (DMSO). Control groups included one group of vehicle alone, and one group of (DMSO) at final concentration of 1%.

Each dose group contained 10 mice. Mice were injected subcutaneously (scruff of neck) with 0.5 ml of the appropriate sample. Forty eight hours following the sample injection, the mice were administered an intraperitoneal injection of 0.2 ml of [<sup>59</sup>Fe]

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1 (approximately 18.0 milliCuries/milligram, Dupont, NEN)  
and 0.75 microCuries/Mouse.

5 Mouse body weights were determined twenty four  
hours following [ $^{59}\text{Fe}$ ] administration and the mice were  
sacrificed forty eight hours following the [ $^{59}\text{Fe}$ ]  
injection. Blood was collected from each animal by  
cardiac puncture and hematocrits were determined  
(heparin was used as the anticoagulant). Each blood  
10 sample (0.2 ml) was analyzed for [ $^{59}\text{Fe}$ ] incorporation  
using a Packard gamma counter. Non-responder mice  
(i.e., those mice with radioactive incorporation less  
than the negative control group) were eliminated from  
the appropriate data set. Mice that had hematocrit  
values less than 53% were also eliminated.

15 This assay examined the ability of an  
exogenously administered compound to induce new red  
blood cell synthesis, or in other words to function as  
EPO or an EPO mimetic. The results are derived from  
sets of 10 animals for each experimental dose. As shown  
20 in Figure 7 and Table IV, the data suggests that on a  
mole equivalent basis, peptide (SEQ ID NO: 8)/PEG-dimer  
is about 10 fold more active than peptide (SEQ ID NO: 8)  
monomer. These results are consistent with in vitro  
results in which increased potency values of 10 fold was  
25 observed on murine EPO-R bearing cells.

**TABLE IV**

30 **Table 4. Exhpoic Mouse Bioassay Study of PEG Dimer Activity**

Compound	Amount required for equivalency to 0.025U EPO (nmol) n=10
Seq. I.D. No. 8	3.8

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EXAMPLE 14

This example shows that an inactive truncation analog of peptide (SEQ ID NO: 8), which lacks the critical tyrosine peptide (SEQ ID NO: 18), (SCHFGPLTWVCK), can be converted to an agonist on the human EPO receptor cell line by PEG dimerization. In this experiment, a  $10^{-5}$  M concentration of the parent peptide had no activity above background while the dimeric peptide exhibited a level of proliferation twice as many cpm as background. As shown in Figure 8, the peptide alone (open squares) did not induce proliferation of the EPO responsive cells but upon PEG dimerization (open diamonds) a significant agonist effect was observed. Approximately twice as many cpm incorporated over non-stimulated cells at  $10^{-5}$  M added peptide dimer. The replicate error bars represent the standard deviation of three assay points per concentration of peptide or peptide dimer.

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1 Exhypoxic polycythemic mouse bioassay:

PEG dimer and the monomer parent peptide RWJ 61718 were compared in the exhypoxic mouse bioassay (Table 1). This peptide exhibited an  
5 80 fold increase in in vitro activity upon dimerization. Murine studies (in vivo) of the activity of the dimer compared to the monomer peptide revealed a 250 fold increase in activity of RWJ 61718 upon dimerization. Cellular proliferation studies on this dimer peptide in murine receptor containing cells demonstrated a 16  
10 fold increase over the monomer indicating that the 250 fold increase in vivo might be attributable to other factors such as altered metabolism or prolonged circulatory half-life which occur upon PEG dimerization of the peptide sequence. Thus, in addition to the effect of dimerization alone, the PEG modification has an effect which impacts in vivo activity and may be specific to individual  
15 peptide sequences.

Cell associated EPO receptor competition binding assay.

A competitive binding analysis of the ability of selected monomer peptides and their cognate dimer products to compete with  
20 radiolabelled EPO binding for cell associated human EPO receptors was performed Erythropoietin Receptor Competition Binding Analysis was performed as follows. TF-1 cells were maintained in RPMI 1640, 10% fetal calf serum, 1% L-glutamine, 1% penicillin, 0.1% streptomycin and 1 ng/ml of GM-CSF. [125]-EPO was obtained from NEN  
25 Research Products. Cells were centrifuged and washed 1 x with binding buffer (RPMI 1640, 5% BSA, 25 mM Hepes, pH 7.5, 0.02% sodium azide) resuspended in binding buffer, and counted using trypan blue as an indicator of viability. Each reaction contained approximately  $5 \times 10^5$  cells, [125]-EPO (0.5 nM), no competitor or peptide or dimer preparation in a final volume of 200  $\mu$ l. The binding reactions (in  
30 duplicate) were incubated overnight at 4°C. Following binding, the tubes were centrifuged at 12,000 rpm for 1 min at 4°C in a

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1 refrigerated centrifuge. The supernatant was removed, the cell  
pellet resuspended in 100 µl of binding buffer, and the cell  
suspension layered onto 0.7 ml of bovine calf serum. The tubes were  
centrifuged at 12,000 rpm for 5 min at 4°C, the supernatant was  
5 removed, the bottom of the tubes snipped off, and the cell pellets  
counted in a Micromedic ME plus gamma counter. Non-specific binding  
was determined by incubating cells with [125]-EPO and a 100-fold  
excess of non-radioactive EPO. These data demonstrate increases in  
apparent binding competitive affinity of 3.0 fold, 3.2 fold and 80  
10 fold for peptides RWJ 61233, RWJ 61596 and RWJ 61718, respectively  
(Table 2). In vivo proliferation studies with these peptides and  
their dimer derivatives reveal increases in potency of ea. 50 fold,  
10 fold and 80 fold, respectively, indicating that the magnitude of  
increased binding affinity is exceeded by the functional potency of  
the peptide for two of the three species. Thus, the effect of  
15 dimerization and subsequent increase in activity may be one in which  
the efficiency of receptor stimulation is improved by limiting the  
lateral diffusion of the receptors away from a binding event.  
Peptide dimerization therefore likely results in entropic rather  
than enthalpic gains upon mimetic ligand-receptor association for  
some peptide dimer sequences.

20 Unlike the EBP-bead EPO competitive binding assay where peptide  
dimerization negatively impacted the ability of PEG dimer peptides  
to compete for receptor binding, the ability to compete for cell  
associated receptors is increased by dimerization. This may be due  
25 to the ability of the cell associated receptor to dimerize while the  
immobilized EBP monomer likely cannot.

Conversion of inactive to active peptide RWJ 61177 was further  
studied. An improved and expanded study was performed which  
confirmed our earlier observation of conversion to an active peptide  
30 (Figure 6, Panel D).

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TABLE IV. EXHYPOXIC MOUSE BIOASSAY STUDY OF PEG DIMER ACTIVITY

Compound	Amount required for equivalency to 0.025 U EPO (nmol) n=10
RWJ 61233 (seq. ID #8)	3.8
SAP2/61233, covalent dimer	0.28
RWJ 61718 (seq. ID #14)	18
SAP2/61718, covalent dimer	0.07



TABLE V. EPO COMPETITIVE BINDING ANALYSIS OF CELL ASSOCIATED RECEPTORS

Compound	IC <sub>50</sub> (μM)
RWJ 61233 (seq ID #8)	3.8
SAP2/61233, covalent dimer	0.28
RWJ 61718 (seq ID#14)	18
SAP2/61718, covalent dimer	0.07

## SEQUENCE LISTING

## GENERAL INFORMATION:

- i) APPLICANT: Johnson, Dana L  
Zivin, Robert A
- ii) TITLE OF INVENTION: AGONIST PEPTIDE DIMERS
- iii) NUMBER OF SEQUENCES: 93
- iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Frank S. DiGiglio
  - (B) STREET: 400 Garden City Plaza
  - (C) CITY: Garden City
  - (D) STATE: New York
  - (E) COUNTRY: U.S.A..
  - (F) ZIP: 11530
- v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/484,135
  - (B) FILING DATE: 07-JUN-1995
  - (C) CLASSIFICATION:
- vii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: DiGiglio, Frank S
  - (B) REGISTRATION NUMBER: 31,346
  - (C) REFERENCE/DOCKET NUMBER: 9594
- viii) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (516) 742-4343
  - (B) TELEFAX: (516) 742-4366

## INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 10 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

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## c) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc; Xaa(Pos2) can be R,H,L or W; Xaa(Pos3) can be M,F or I; Xaa(Pos6) can be any one of the 20 L-amino acids or the stereoisomeric D-amino acids; Xaa(Pos9) can be D,E,I,L or V; and Xaa(Pos10) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc, provided that either Xaa(Pos1) or Xaa(Pos10) is C or Hoc"

## i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa  
                   5                  10

## INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "Xaa(Pos2) and Xaa(Pos8) can be any one of the 20 L-amino acids; Xaa(Pos3) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc; Xaa(Pos4) can be R,H,L or W; Xaa(Pos5) can be M,F or I; Xaa(Pos11) can be D,E,I,L or V; and Xaa(Pos12) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc, provided that either Xaa(Pos3) or Xaa(Pos12) is C or Hoc"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Tyr Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa  
   1                  5                  10

## INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

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## :) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..16
- (D) OTHER INFORMATION: /note= "Xaa(Pos1), Xaa(Pos 3), Xaa(Pos9), Xaa(Pos14), Xaa(Pos15) and Xaa(Pos16) can be any one of 20 L-amino acids; Xaa(Pos4) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc; Xaa(Pos5) can be R,H,L or W; Xaa(Pos6) can be M,F or I; Xaa(Pos12) can be D,E,I,L or V; and Xaa(Pos13) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc, provided that either Xaa(Pos4) or Xaa(Pos13) is C or Hoc"

## i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

aa Tyr Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa Xaa Xaa Xaa
   5                               10                           15

```

## INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Xaa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa
 1           5                               10                           15

```

## INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..16
- (D) OTHER INFORMATION: /note= "Xaa(Pos1), Xaa(Pos3) and Xaa(Pos16) can be any one of the 20 L-amino acids; Xaa(Pos5) can be R or H; Xaa(Pos6) can be F or M; Xaa(Pos9) can be I,L,T,M or V; Xaa(Pos12) can be D or V; Xaa(Pos14) can be G,T,L,Q,R,S or T; Xaa(Pos15) can be A,G,P,R or Y"

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## i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

aa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa  
                   5                                  10                                  15

## FORMATION FOR SEQ ID NO:6:

## i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## x) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..16
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be D,E,L,N,S,T or V;  
 Xaa(Pos3) can be A,H,K,L,M,S or T; Xaa(Pos5) can be R or H;  
 Xaa(Pos6) can be F or M; Xaa(Pos9) can be I,L,T,M or V;  
 Xaa(Pos12) can be D or V; Xaa(Pos14) can be K,R,S or T;  
 Xaa(Pos15) is P and Xaa(Pos16) can be any one of the 20 L-amino  
 acids"

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Tyr Xaa Cys Xaa Xaa Gly Pro Xaa Thr Trp Xaa Cys Xaa Xaa Xaa  
   1                  5                                  10                                  15

## INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Gly Leu Tyr Leu Cys Arg Phe Gly Pro Val Thr Trp Asp Cys Gly  
   1                  5                                  10                                  15

Tyr Lys Gly Gly  
                   20

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## FORMATION FOR SEQ ID NO:8:

## .) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys  
                  5                  10                  15  
ro Gln Gly Gly  
                  20

## FORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Gly Asp Tyr His Cys Arg Met Gly Pro Leu Thr Trp Val Cys Lys  
1                  5                  10                  15  
Pro Leu Gly Gly  
                  20

## FORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys  
                   5                                  10                                  15

ro Gln

FORMATION FOR SEQ ID NO:14:

- i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Gly Leu Tyr Ala Cys His Met Gly Pro Met Thr Trp Val Cys Gln  
   1                  5                                  10                                  15

Pro Leu Arg Gly  
                   20

INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Thr Ile Ala Gln Tyr Ile Cys Tyr Met Gly Pro Glu Thr Trp Glu Cys  
   1                  5                                  10                                  15

Arg Pro Ser Pro Lys Ala  
                   20



## FORMATION FOR SEQ ID NO:16:

## i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

## i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

hr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys  
5 10

## FORMATION FOR SEQ ID NO:17:

## i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Tyr Cys His Phe Gly Pro Leu Thr Trp Val Cys  
1 5 10

## FORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys  
1 5 10

## INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids

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- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

c) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "Xaa(Pos1) can be any one of the 20 L-amino acids; except that Xaa(Pos1) may or may not be Y and Xaa(Pos1) may be any non-naturally occurring aromatic acid analog when Xaa(Pos1) is Y. Xaa(Pos2) and Xaa(Pos8) can be any one of the 20 L-amino acids; Xaa(Pos3) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc; Xaa(Pos4) can be R,H,L or W; Xaa(Pos5) can be M,F or I; Xaa(Pos11) can be D,E,I,L or V and Xaa(Pos12) can be C,A, $\alpha$ -amino- $\gamma$ -bromobutyric acid or Hoc provided that either Xaa(Pos3) or Xaa(Pos12) is C or Hoc"

ii) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Xaa Xaa Xaa Xaa Xaa Gly Pro Xaa Thr Trp Xaa Xaa  
                                   5                                  10

INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys  
   1                  5                                  10                                  15  
 Pro Gln Gly Gly  
                                   20

INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ly Gly Thr Tyr Arg Cys Ser Met Gly Pro Met Thr Trp Val Cys Leu  
5 10 15  
ro Met Gly Gly  
20

FORMATION FOR SEQ ID NO:22:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gly Gly Met Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Gly  
1 5 10 15  
Pro Ser Gly Gly  
20

FORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Gly Trp Ala Trp Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser  
1 5 10 15  
Ala His Gly Gly  
20

## FORMATION FOR SEQ ID NO:24:

- i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Gly Met Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Ile  
1                    5                    10                    15

Pro Tyr Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Gly Glu Tyr Lys Cys Tyr Met Gly Pro Ile Thr Trp Val Cys Lys  
1                    5                    10                    15

Pro Glu Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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## i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ly Gly Asp Tyr Thr Cys Arg Met Gly Pro Met Thr Trp Ile Cys Thr  
                   5                  10                  15  
 .la Thr Gly Gly  
                   20

## INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Gly Asn Tyr Leu Cys Arg Phe Gly Pro Gly Thr Trp Asp Cys Thr  
 1                  5                  10                  15  
 Gly Phe Arg Gly  
                   20

## INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gly Gly Asn Tyr Val Cys Arg Met Gly Pro Ile Thr Trp Ile Cys Thr  
 1                  5                  10                  15  
 Pro Ala Gly Gly  
                   20

## INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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- (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Gly Lys Asp Val Cys Arg Met Gly Pro Ile Thr Trp Asp Cys Arg  
                   5                  10                  15  
 Ser Thr Gly Gly  
                   20

INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 23 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Gly Ser Tyr Leu Cys Arg Met Gly Pro Thr Thr Trp Leu Cys Thr  
   1                  5                  10                  15  
 Ala Gln Arg Gly Gly Gly Asn  
                   20

INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly Gly Asn Tyr Leu Cys Arg Met Gly Pro Ala Thr Trp Val Cys Gly  
   1                  5                  10                  15  
 Arg Met Gly Gly  
                   20

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## FORMATION FOR SEQ ID NO:32:

## i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gly Gly Glu Tyr Lys Cys Arg Met Gly Pro Leu Thr Trp Val Cys Gln  
1                      5                      10                      15  
Tyr Ala Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:33:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Gly Asp Tyr Thr Cys Arg Met Gly Pro Met Thr Trp Ile Cys Thr  
1                      5                      10                      15  
Ala Thr Arg Gly  
                    20

## INFORMATION FOR SEQ ID NO:34:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

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## (i) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Gly Val Tyr Val Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Thr  
1 5 10 15  
Ala Ser Gly Gly  
20

## INFORMATION FOR SEQ ID NO:35:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Gly Glu Tyr Ser Cys Arg Met Gly Pro Met Thr Trp Val Cys Ser  
1 5 10 15  
Pro Thr Gly Gly  
20

## INFORMATION FOR SEQ ID NO:36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Gly Glu Tyr Leu Cys Arg Met Gly Pro Ile Thr Trp Val Cys Glu  
1 5 10 15  
Arg Tyr Gly Gly  
20

## INFORMATION FOR SEQ ID NO:37:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid



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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ly Gly Asn Tyr Ile Cys Arg Met Gly Pro Met Thr Trp Val Cys Thr  
                   5                  10                  15  
 la His Gly Gly  
                   20

INFORMATION FOR SEQ ID NO:38:

i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Gly Asp Tyr Leu Cys Arg Met Gly Pro Ala Thr Trp Val Cys Gly  
 1                  5                  10                  15  
 Arg Met Gly Gly  
                   20

INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Gly Gly Leu Tyr Leu Cys Arg Phe Gly Pro Val Thr Trp Asp Cys Gly  
 1                  5                  10                  15  
 Tyr Lys Gly Gly  
                   20

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## FORMATION FOR SEQ ID NO:40:

## i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## i) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ly Gly Leu Tyr Ser Cys Arg Met Gly Pro Ile Thr Trp Val Cys Thr  
5 10 15

ys Ala Gly Gly  
20

## FORMATION FOR SEQ ID NO:41:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gly Gly Gly Tyr His Cys Arg Met Gly Pro Met Thr Trp Val Cys Arg  
1 5 10 15

Pro Val Gly Gly  
20

## FORMATION FOR SEQ ID NO:42:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

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) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Y Gly Thr Tyr Ser Cys His Phe Gly Pro Leu Thr Trp Val Cys Lys  
                   5                  10                  15  
 Pro Gln Gly Gly  
                   20

FORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Gly Ile Tyr Lys Cys Leu Met Gly Pro Leu Thr Trp Val Cys Thr  
                   5                  10                  15  
 Pro Asp Gly Gly  
                   20

FORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Gly Leu Tyr Ser Cys Leu Met Gly Pro Ile Thr Trp Leu Cys Lys  
   1                  5                  10                  15  
 Pro Lys Gly Gly  
                   20

FORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

- (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gly Asp Tyr His Cys Arg Met Gly Pro Leu Thr Trp Val Cys Lys  
                   5                                  10                                  15  
 Leu Gly Gly  
                   20

FORMATION FOR SEQ ID NO:46:

- i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ly Gly Asp Tyr Ser Cys Arg Met Gly Pro Thr Thr Trp Val Cys Thr  
                   5                                  10                                  15  
 ro Pro Gly Gly  
                   20

FORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gly Gly Asp Tyr Trp Cys Arg Met Gly Pro Ser Thr Trp Glu Cys Asn  
   1                  5                                  10                                  15  
 Ala His Gly Gly  
                   20

## FORMATION FOR SEQ ID NO:48:

## i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Gly Gly Lys Tyr Leu Cys Ser Phe Gly Pro Ile Thr Trp Val Cys Ala  
1                      5                      10                      15  
Arg Tyr Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:49:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gly Gly Leu Tyr Lys Cys Arg Leu Gly Pro Ile Thr Trp Val Cys Ser  
1                      5                      10                      15  
Pro Leu Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:50:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (i) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Gly Gly Ser Tyr Thr Cys Arg Phe Gly Pro Glu Thr Trp Val Cys Arg  
5 10 15  
Pro Asn Gly Gly  
20

## INFORMATION FOR SEQ ID NO:51:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Gly Ser Tyr Ser Cys Arg Met Gly Pro Ile Thr Trp Val Cys Lys  
1 5 10 15  
Pro Gly Gly Gly  
20

## INFORMATION FOR SEQ ID NO:52:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gly Gly Ser Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu  
1 5 10 15  
Pro Ala Gly Gly  
20

## INFORMATION FOR SEQ ID NO:53:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i) MOLECULE TYPE: peptide

(ii) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Gly	Gly	Leu	Tyr	Glu	Cys	Arg	Met	Gly	Pro	Met	Thr	Trp	Val	Cys	Arg
1				5				10						15	
Pro Gly Gly Gly															
20															

INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly	Gly	Asp	Tyr	Thr	Cys	Arg	Met	Gly	Pro	Ile	Thr	Trp	Ile	Cys	Thr
1				5				10						15	
Lys Ala Gly Gly															
20															

INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Gly	Gly	Val	Tyr	Ser	Cys	Arg	Met	Gly	Pro	Thr	Thr	Trp	Glu	Cys	Asn
1				5				10						15	
Arg Tyr Val Gly															
20															

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## INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Gly Gly Ala Tyr Leu Cys His Met Gly Pro Ile Thr Trp Val Cys Arg  
1                    5                    10                    15  
Pro Gln Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Gly Gly Glu Tyr Ser Cys Arg Met Gly Pro Asn Thr Trp Val Cys Lys  
1                    5                    10                    15  
Pro Val Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide



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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ly Gly Val Tyr Lys Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Arg  
                   5                                  10                                  15  
 ro Thr Gly Gly  
                   20

INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Gly Gly Asp Tyr Asn Cys Arg Phe Gly Pro Leu Thr Trp Val Cys Lys  
 1                  5                                  10                                  15  
 Pro Ser Gly Gly  
                   20

INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Gly Gly Ser Tyr Leu Cys Arg Phe Gly Pro Thr Thr Trp Leu Cys Ser  
 1                  5                                  10                                  15  
 Ser Ala Gly Gly  
                   20

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## 1) SEQUENCE DESCRIPTION: SEQ ID NO:58:

ly Gly Leu Tyr Leu Cys Arg Met Gly Pro Val Thr Trp Glu Cys Gln  
                   5                                  10                                  15  
 ro Arg Gly Gly  
                   20

## FORMATION FOR SEQ ID NO:59:

## i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## .i) MOLECULE TYPE: peptide

## :i) SEQUENCE DESCRIPTION: SEQ ID NO:59:

gly Gly Leu Tyr Thr Cys Pro Met Gly Pro Ile Thr Trp Val Cys Leu  
 1                  5                                  10                                  15  
 Leu Pro Gly Gly  
                   20

## NFORMATION FOR SEQ ID NO:60:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Val Thr Trp Val Cys Thr  
 1                  5                                  10                                  15  
 Gly Ala Gly Gly  
                   20

## INFORMATION FOR SEQ ID NO:61:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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## i) SEQUENCE DESCRIPTION: SEQ ID NO:66:

ly Gly Trp Val Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gly  
                   5                  10                  15  
 al His Gly Gly  
                   20

## INFORMATION FOR SEQ ID NO:67:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Gly Gly Gln Leu Leu Cys Gly Ile Gly Pro Ile Thr Trp Val Cys Arg  
 1                  5                  10                  15  
 Trp Val Gly Gly  
                   20

## INFORMATION FOR SEQ ID NO:68:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gly Gly Lys Tyr Ser Cys Phe Met Gly Pro Thr Thr Trp Val Cys Ser  
 1                  5                  10                  15  
 Pro Val Gly Arg Gly Val  
                   20

## INFORMATION FOR SEQ ID NO:69:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

MOLECULE TYPE: peptide

SEQUENCE DESCRIPTION: SEQ ID NO:77:

/ Gly Leu Tyr Leu Cys Arg Met Gly Pro Gln Thr Trp Met Cys Gln  
                   5                                  10                                  15

o Gly Gly Gly  
                   20

FORMATION FOR SEQ ID NO:78:

.) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:78:

ly Gly Asp Tyr Val Cys Arg Met Gly Pro Met Thr Trp Val Cys Ala  
                   5                                  10                                  15

ro Tyr Gly Arg  
                   20

FORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Gly Gly Trp Tyr Ser Cys Leu Met Gly Pro Met Thr Trp Val Cys Lys  
   1                  5                                  10                                  15

Ala His Arg Gly  
                   20

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## FORMATION FOR SEQ ID NO:80:

- i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

ci) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Gly Gly Lys Tyr Tyr Cys Trp Met Gly Pro Met Thr Trp Val Cys Ser  
1                      5                      10                      15  
Pro Ala Gly Gly  
                    20

## NFORMATION FOR SEQ ID NO:81:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Gly Gly Tyr Val Met Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Asp  
1                      5                      10                      15  
Ile Pro Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

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## ii) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Gly Ser Cys Leu Gln Cys Cys Ile Gly Pro Ile Thr Trp Val Cys Arg  
5 10 15  
His Ala Gly Gly  
20

## INFORMATION FOR SEQ ID NO:83:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## ii) MOLECULE TYPE: peptide

## xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Gly Gly Asn Tyr Phe Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gln  
1 5 10 15  
Arg Ser Val Gly  
20

## INFORMATION FOR SEQ ID NO:84:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Gly Gly Glu Tyr Ile Cys Arg Met Gly Pro Leu Thr Trp Glu Cys Lys  
1 5 10 15  
Arg Thr Gly Gly  
20

## INFORMATION FOR SEQ ID NO:85:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Gly Gly Leu Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Lys  
 1                      5                      10                      15  
 Tyr Met Ala Gly  
                     20

INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Gly Gly Gln Tyr Leu Cys Thr Phe Gly Pro Ile Thr Trp Leu Cys Arg  
 1                      5                      10                      15  
 Gly Ala Gly Gly  
                     20

INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Gly Gly Val Tyr Ala Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser  
 1                      5                      10                      15  
 Pro Leu Gly Gly  
                     20

## INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Gly Gly Tyr Thr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Ser  
1                    5                    10                    15  
Ala His Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Gly Gly Thr Tyr Lys Cys Trp Met Gly Pro Met Thr Trp Val Cys Arg  
1                    5                    10                    15  
Pro Val Gly Gly  
                    20

## INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide



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.) SEQUENCE DESCRIPTION: SEQ ID NO:90:

ly Gly Asn Tyr Tyr Cys Arg Phe Gly Pro Ile Thr Phe Glu Cys His  
5 10 15  
ro Thr Gly Gly  
20

FORMATION FOR SEO ID NO:91:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

.i) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Gly Gly Glu Tyr Leu Cys Arg Met Gly Pro Asn Thr Trp Val Cys Thr  
1 5 10 15  
Pro Val Gly Gly  
20

INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu  
1 5 10 15  
Pro Ala Gly Gly  
20

INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Gly Gly Leu Tyr Thr Cys Arg Met Gly Pro Ile Thr Trp Val Cys Leu  
1                      5                      10                      15

Pro Ala Gly Gly  
20

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Gly Gly Trp Val Tyr Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Asp  
1 5 10 15  
Thr Asn Gly Gly  
20

INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Gly Gly Met Tyr Tyr Cys Arg Met Gly Pro Met Thr Trp Val Cys Lys  
1 5 10 15  
Gly Ala Gly Gly  
20

INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Gly Gly Thr Thr Gln Cys Trp Ile Gly Pro Ile Thr Trp Val Cys Arg  
1 5 10 15  
Ala Arg Gly Gly  
20

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## FORMATION FOR SEQ ID NO:72:

- i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

ii) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Gly Gly Pro Tyr His Cys Arg Met Gly Pro Ile Thr Trp Val Cys Gly  
                  5                                  10                                  15

Pro Val Gly Gly  
                  20

## FORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 20 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Gly Gly Glu Tyr Arg Cys Arg Met Gly Pro Ile Ser Trp Val Cys Ser  
1                  5                                  10                                  15

Pro Gln Gly Gly  
                  20

## FORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 22 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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) SEQUENCE DESCRIPTION: SEQ ID NO:74:

y Gly Asn Tyr Thr Cys Arg Phe Gly Pro Leu Thr Trp Glu Cys Thr  
                   5                  10                  15

o Gln Gly Gly Gly Ala  
                   20

FORMATION FOR SEQ ID NO:75:

.) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: peptide

i) SEQUENCE DESCRIPTION: SEQ ID NO:75:

ly Gly Ser Trp Asp Cys Arg Ile Gly Pro Ile Thr Trp Val Cys Lys  
                   5                  10                  15

rp Ser Gly Gly  
                   20

FORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Val Gly Asn Tyr Met Cys His Phe Gly Pro Ile Thr Trp Val Cys Arg  
   1                  5                  10                  15

Pro Gly Gly Gly  
                   20

FORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid

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1 WE CLAIM:

5 1. A. peptide dimer comprising two monomeric peptides of 10 to about 40 amino acids in length that bind to EPO receptor, each monomeric peptide comprising a sequence of amino acids  $X_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 1) wherein  $X_6$  is selected from any of the 20 genetically coded L-amino acids;  $X_3$  is C;  $X_4$  is R, H, L or W;  $X_5$  is M, F or I;  $X_7$  is D, E, I, L or V; and  $X_8$  is C.

10 2. The peptide dimer of Claim 1 wherein each of said monomeric peptides comprise a sequence of amino acids  $YX_2X_3X_4X_5GPX_6TWX_7X_8$ , (SEQ ID NO: 2) wherein each of  $X_2$  and  $X_6$  is independently selected from any one of the 20 genetically coded L-amino acids;  $X_3$  is C;  $X_4$  is R, H, L or W;  $X_5$  is M, F or I;  $X_7$  is D, E, I, L or V; and  $X_8$  is C.

15 3. The peptide dimer of Claim 2 wherein each of said monomeric peptides comprise a sequence of amino acids  $X_1YX_2X_3X_4X_5GPX_6TWX_7X_8X_9X_{10}X_{11}$  (SEQ ID NO: 3) wherein each of  $X_1$ ,  $X_2$ ,  $X_6$ ,  $X_9$ ,  $X_{10}$ , and  $X_{11}$  is independently selected from any one of the 20 genetically coded L-amino acids;  $X_3$  is C;  $X_4$  is R, H, L or W;  $X_5$  is M, F or I;  $X_7$  is D, E, I, L or V; and  $X_8$  is C.

20 4. The peptide dimer of Claim 3 wherein  $X_4$  is R or H;  $X_5$  is F or M;  $X_6$  is I, L, T, M or V;  $X_7$  is D or V;  $X_9$  is G, K, L, Q, R, S, or T; and  $X_{10}$  is A, G, P, R, or Y.

25 5. The peptide dimer of Claim 4 wherein  $X_1$  is D, E, L, N, S, T or V;  $X_2$  is A, H, K, L, M, S, or T;  $X_4$  is R or H;  $X_6$  is K, R, S, or T; and  $X_{10}$  is P.

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1           6. The peptide dimer of Claim 1 wherein said  
monomeric peptides are

5           GGLYLCRFGPVTWDCGYKGG       (SEQ ID NO: 7);  
          GGTYSCHFGPLTWVCKPQGG       (SEQ ID NO: 8);  
          GGDYHCRMGPPLTWVCKPLGG       (SEQ ID NO: 9);  
          VGNYMCHFGPITWVCRPGGG       (SEQ ID NO: 10);  
          GGVYACRMGPITWVCSPLGG       (SEQ ID NO: 11);  
          VGNYMAHMGPIWVCRPGG        (SEQ ID NO: 12);  
0           GGTYSCHFGPLTWVCKPQ        (SEQ ID NO: 13);  
          GGLYACHMGPMTWVCQPLRG       (SEQ ID NO: 14);  
          TIAQYICYMGPETWECRPSPKA     (SEQ ID NO: 15);  
          YSCHFGPLTWVCK               (SEQ ID NO: 16);  
          YCHFGPLTWVC                 (SEQ ID NO: 17); and  
          SCHFGPLTWVCK                (SEQ ID NO: 18)

5           7. A pharmaceutical composition comprising at  
least one peptide dimer of any one of Claims 1-6.

20          8. A method for treating a patient having a  
disorder characterized by a deficiency of EPO or low or  
defective red blood cell population comprising  
administering to said patient a therapeutically  
effective amount of at least one peptide dimer of any  
one of Claims 1-6.

25          9. The peptide dimer of any one of Claims 1-6  
wherein said dimer is formed by a polyethylene glycol  
linker through a covalent bond.

30          10. The peptide dimer of any one of Claims 1-  
6 wherein said monomeric peptide units are dimerized on  
activated benodiazepins, oxazolones, azalactones,  
aminimides or diketopiperazine.

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1           11. The peptide dimer of Claim 9 wherein said monomeric peptides are covalently bound N-terminus to N-terminus.

5           12. The peptide dimer of Claim 10 wherein said monomeric peptides are covalently bound N-terminus to N-terminus.

          13. The peptide dimer of Claim 9 wherein said monomeric peptides are covalently bound N-terminus to C-terminus.

10          14. The peptide of Claim 10 wherein said monomeric peptides are covalently bound N-terminus to C-terminus.

          15. A method of improving the bioactivity of a cell surface receptor comprising dimerizing a monomeric agonist of said cell surface receptor and contacting said formed dimer with said cell surface receptor to effect said improved biological activity.

15           16. A method of activating a cell surface receptor to induce biological activity of said cell surface receptor comprising dimerizing a monomeric agonist of said cell surface receptor and contacting said formed dimer with said receptor thereby inducing said biological activity.

20           17. The method of Claim 15 or 16 wherein said cell surface receptor is contacted with said dimer in vitro or in vivo.

25           18. The method of Claim 15 or 16 wherein said cell surface receptor is EPO-R.

30           19. The method of Claim 15 or 16 wherein said cell surface agonist is a GH agonist, PDGF agonist, ICF agonist, G-CSF agonist, TPO agonist, VEGF agonist, FGF



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1 agonist, insulin agonist, IL-3 agonist, IL-5 agonist,  
IL-6 agonist or IL-2 agonist.

20. The method of Claim 15 or 16 wherein said  
agonist comprises a sequence of amino acids  
5 YX<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>X<sub>8</sub>, (SEQ ID NO: 2) wherein each of X<sub>2</sub>  
and X<sub>8</sub> is independently selected from any one of the 20  
genetically coded L-amino acids; X<sub>3</sub> is C; X<sub>4</sub> is R, H, L  
or W; X<sub>5</sub> is M, F or I; X<sub>7</sub> is D, E, I, L or V; and X<sub>6</sub> is  
C.

10 21. The method of Claim 15 or 16 wherein said  
agonist comprises a sequence of amino acids  
X<sub>1</sub>YX<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>X<sub>8</sub>X<sub>9</sub>X<sub>10</sub>X<sub>11</sub> (SEQ ID NO: 3) wherein  
each of X<sub>1</sub>, X<sub>2</sub>, X<sub>6</sub>, X<sub>9</sub>, X<sub>10</sub>, and X<sub>11</sub> is independently  
15 selected from any one of the 20 genetically coded L-  
amino acids; X<sub>3</sub> is C; X<sub>4</sub> is R, H, L or W; X<sub>5</sub> is M, F or  
I; X<sub>7</sub> is D, E, I, L or V; and X<sub>8</sub> is C.

22. The method of Claim 15 or 16 wherein said  
agonist comprises a sequence of amino acids  
20 X<sub>1</sub>Y<sub>1</sub>X<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>X<sub>8</sub>X<sub>9</sub>X<sub>10</sub>X<sub>11</sub> (SEQ ID NO: 3) wherein  
each of X<sub>1</sub>, X<sub>2</sub> and X<sub>11</sub> is independently selected from  
any one of the 20 genetically coded L-amino acids; X<sub>3</sub> is  
C; X<sub>4</sub> is R or H; X<sub>5</sub> is F or M; X<sub>6</sub> is I, L, T, M or V; X<sub>7</sub>  
is D or V; X<sub>9</sub> is G, K, L, Q, R, S, or T; and X<sub>10</sub> is A,  
G, P, R, or Y.

25 23. The method of Claims 15 or 16 wherein said  
agonist comprises a sequence of amino acids  
X<sub>1</sub>YX<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>GPX<sub>6</sub>TWX<sub>7</sub>X<sub>8</sub>X<sub>9</sub>X<sub>10</sub>X<sub>11</sub> (SEQ ID NO: 3) wherein X<sub>1</sub>  
is D, E, L, N, S, T or V; X<sub>2</sub> is A, H, K, L, M, S, or T;  
X<sub>3</sub> is C; X<sub>4</sub> is R or H; X<sub>5</sub> is M, F or I; X<sub>6</sub> and X<sub>11</sub> are  
30 independently any one of the 20 genetically coded L-

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1 amino acids; X<sub>7</sub> is D, E, I, L or V; X<sub>8</sub> is C; X<sub>9</sub> is K, R,  
S, or T; and X<sub>10</sub> is P.

24. The method of Claim 15 or 16 wherein said  
agonist is selected from the group consisting of:

5 GGLYLCRFGPVTWDCGYKGG (SEQ ID NO: 7);  
GGTYSCHFGPLTWVCKPQGG (SEQ ID NO: 8);  
GGDYHCRMGPITWVCKPLGG (SEQ ID NO: 9);  
VGNYMCHFGPITWVCRPGGG (SEQ ID NO: 10);  
10 GGVYACRMGPITWVCSPLGG (SEQ ID NO: 11);  
VGNYMAHMGPIWVCRPGG (SEQ ID NO: 12);  
GGTYSCHFGPLTWVCKPQ (SEQ ID NO: 13);  
GGLYACHMGPMPTWVCQPLRG (SEQ ID NO: 14);  
TIAQYICYMGPETWECRPSPKA (SEQ ID NO: 15);  
15 YSCHFGPLTWVCK (SEQ ID NO: 16); and  
YCHFGPLTWVC (SEQ ID NO: 17).

25. The method of Claim 15 or 16 wherein said  
peptide dimers are formed with a polyethylene glycol  
linker through a covalent bond.

26. A method of preparing a cell surface  
receptor agonist comprising dimerizing a cell surface  
antagonist.

27. The method of Claim 26 wherein said cell  
surface antagonist receptor is a GH antagonist, PDGF  
antagonist, EGF antagonist, G-CSF antagonist, EGF  
antagonist, GM-CSF antagonist, TPO antagonist, VEGF  
antagonist, FGF antagonist, insulin antagonist, IL-3  
antagonist, IL-5 antagonist, IL-6 antagonist, or an IL-2  
antagonist.

28. The method of Claim 26 wherein said cell  
surface receptor antagonist is a EPO-R antagonist.

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29. The method of Claim 28 wherein said antagonist comprises a sequence of amino acids  $(AX_2)_nX_3X_4X_5GPX_6TWX_7X_8$  (SEQ ID NO: 19) wherein  $X_6$  is selected from any of the 20 genetically coded L-amino acids;  $X_3$  is C;  $X_4$  is R, H, L or W;  $X_5$  is M, F or I;  $X_7$  is D, E, I, L or V;  $X_8$  is C;  $X_2$  is selected from any of the 20 genetically coded L-amino acids, n is 0 or 1 and A is any of the 20 genetically coded L-amino acids except Y (tyrosine).

30. The method of Claim 21 where said antagonist is SCHFGPLTWVCK (SEQ ID NO: 18).

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## Reverse Phase Analysis of SPA2 Reaction with SEQ. I.D. NO. 8

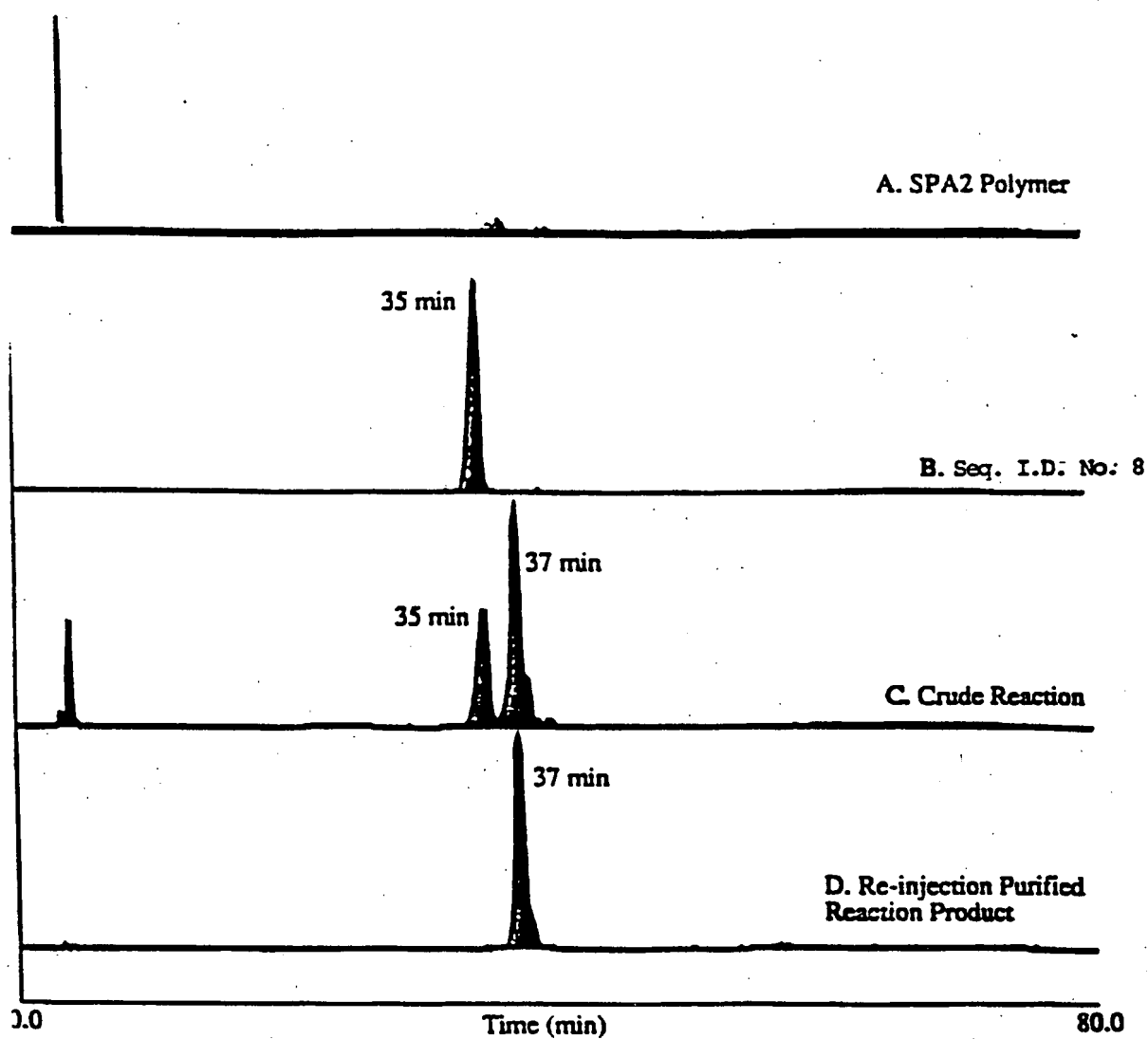


Figure 1

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## Preparative Reverse Phase Analysis of PEG- Peptide (SEQ. I.D. No. 8) Dimer

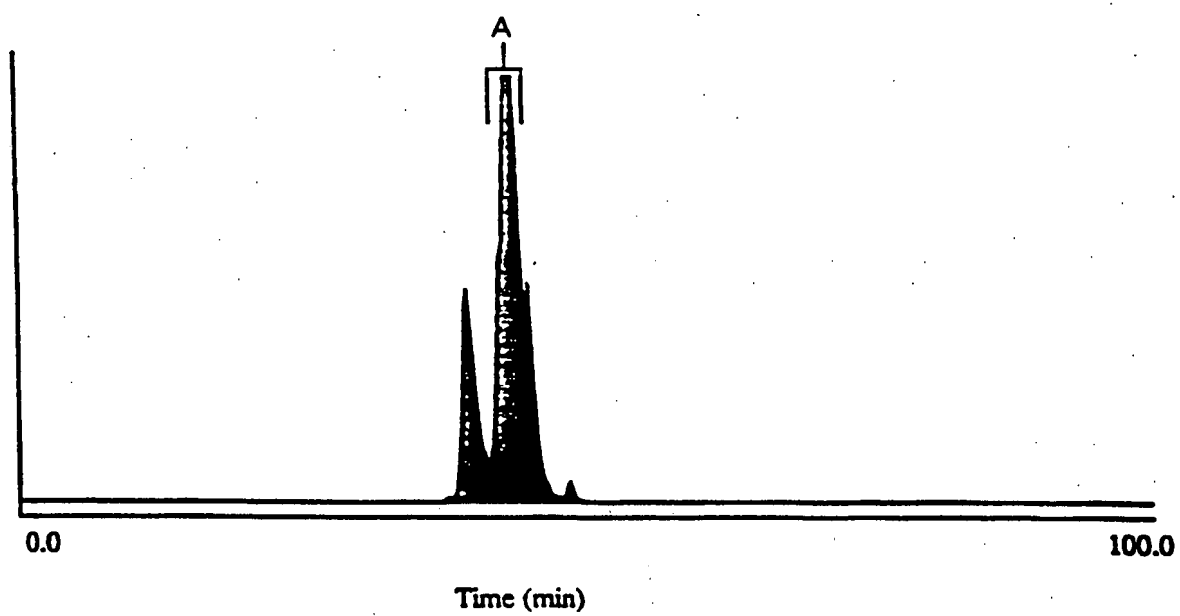
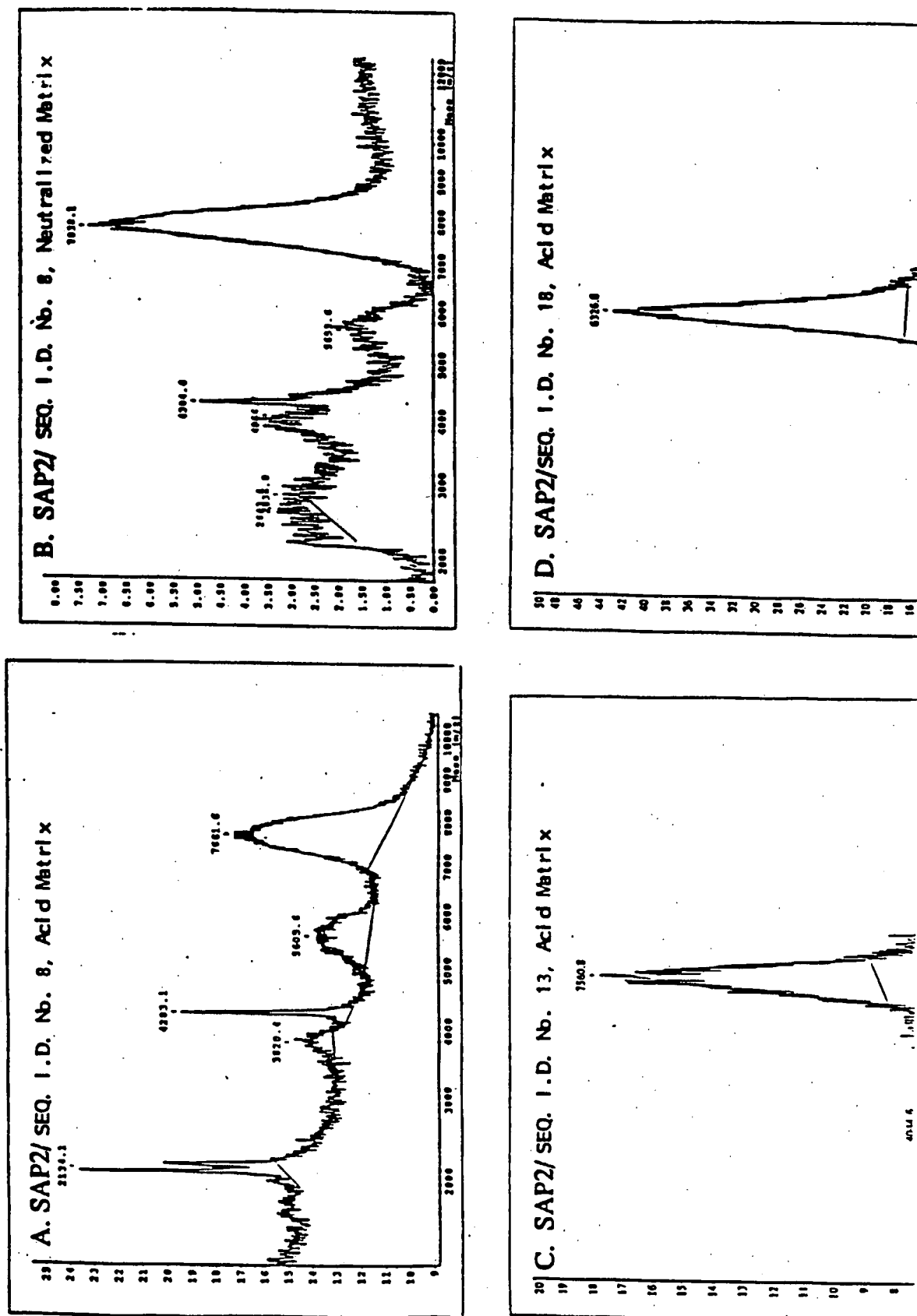


Figure 2

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Figure 3



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A. Non Reducing 10-20% SDS-PAGE. B. Reducing 10-20% SDS-PAGE

Seq. I.D. No.		8		16		18		13		
Lane		3	4	5	6	7	8	9	10	11
1	2									
MWM	EBP	Peptide ( $\mu$ M)	400	400	400	400	400	400	400	---
	EST ( $\mu$ M)	22	22	22	22	22	22	22	22	22
	DPV'S (mM)	1.1	0	1.1	0	1.1	0	1.1	0	1.1

Seq. I.D. No.	Sequence	IC <sub>50</sub> ( $\mu$ M)	EP0-ED <sub>50</sub> ( $\mu$ M)
8	GGTTSCHFGPLTWCKPQGG	5	0.1
16	YSCHFGPLTWCK	70	3
18	SCHFGPLTWCK	90	1A
13	GGTTSCHFGPLTWCKPQ	8	0.08

Figure 4

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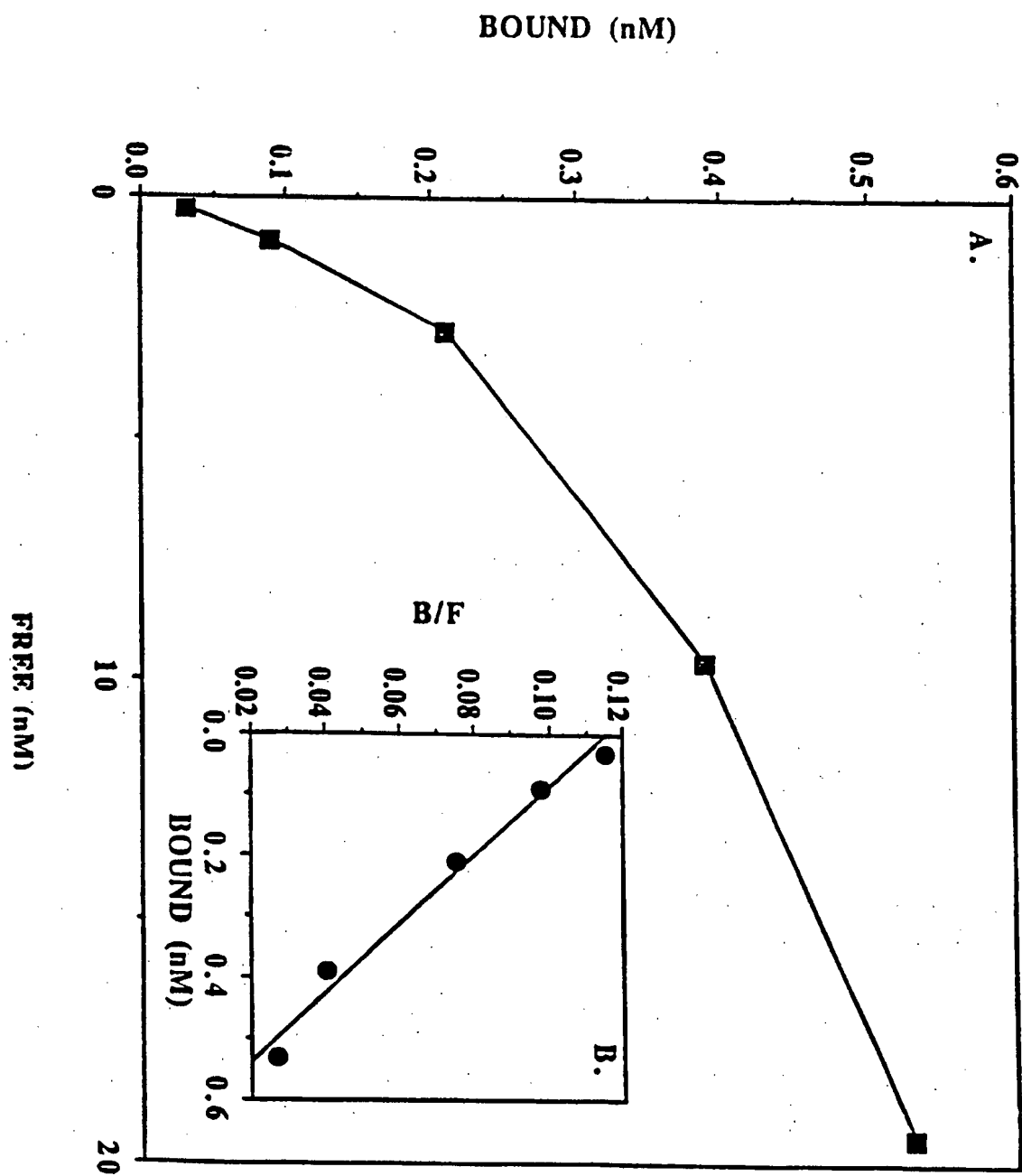
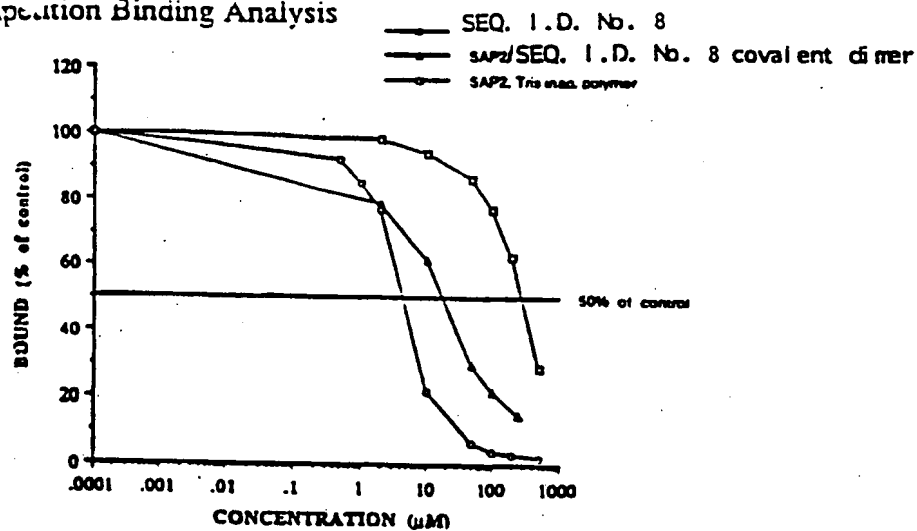


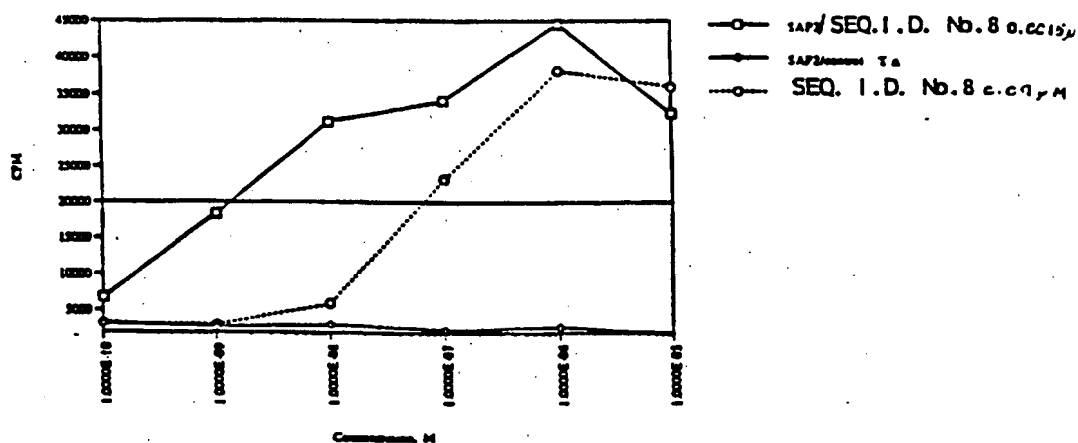
Figure 5



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A.  $[^{125}I]$ EPO Competition Binding Analysis

## B. Cell Proliferation, Human EPOR



## C. Cell Proliferation, Murine EPOR

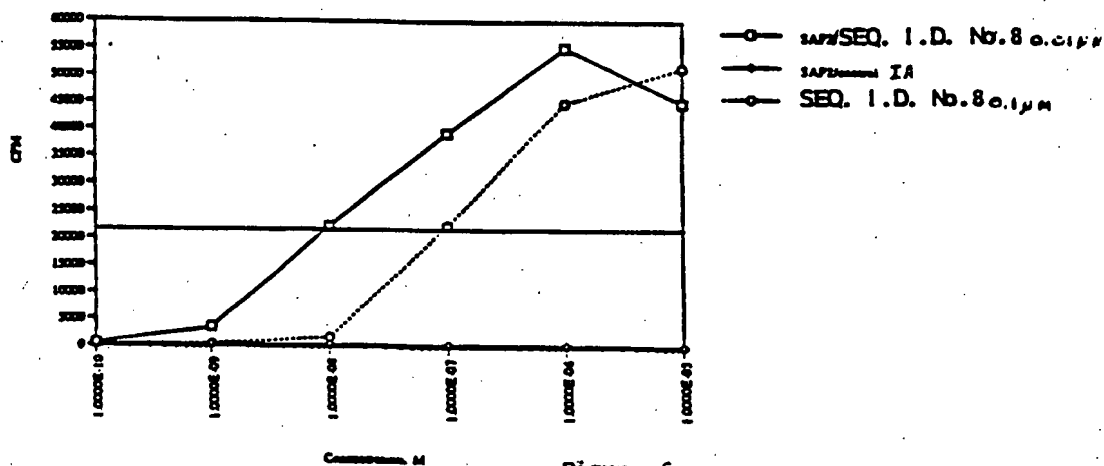


Figure 6

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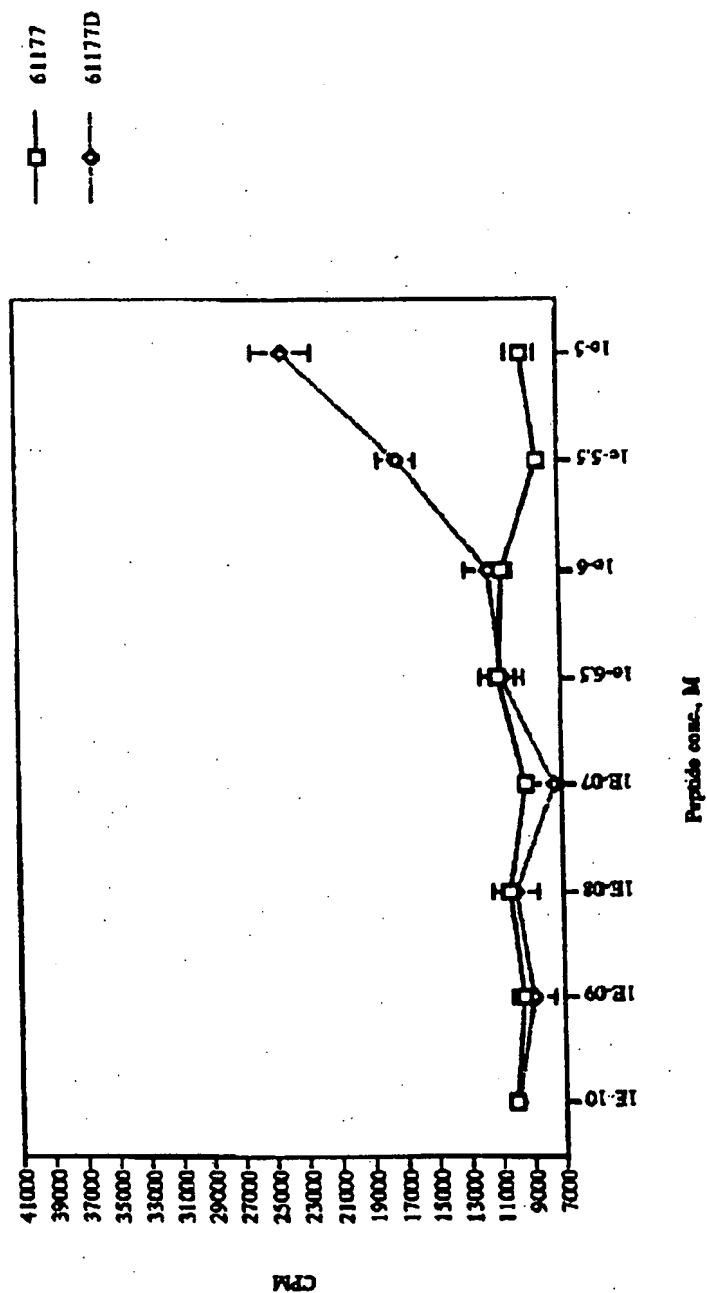


Figure 6  
Panel D

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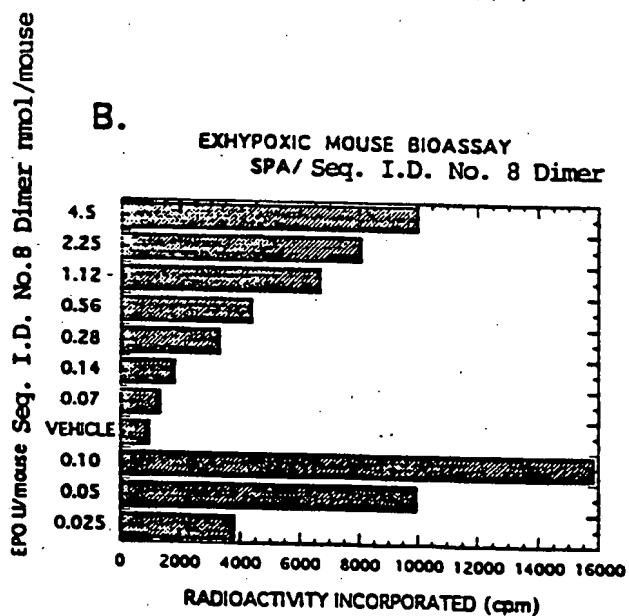
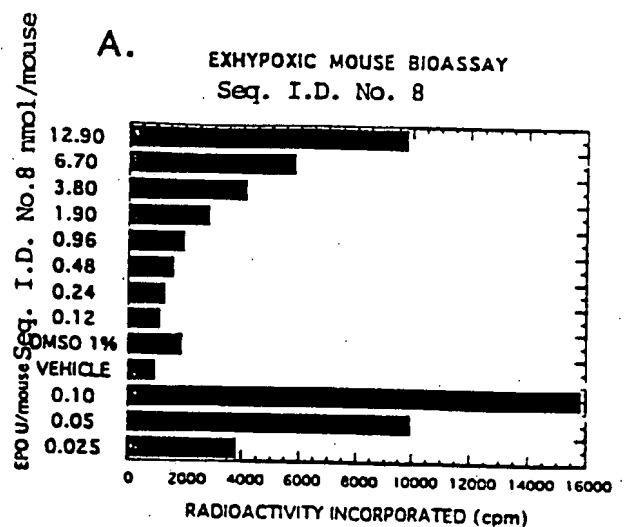
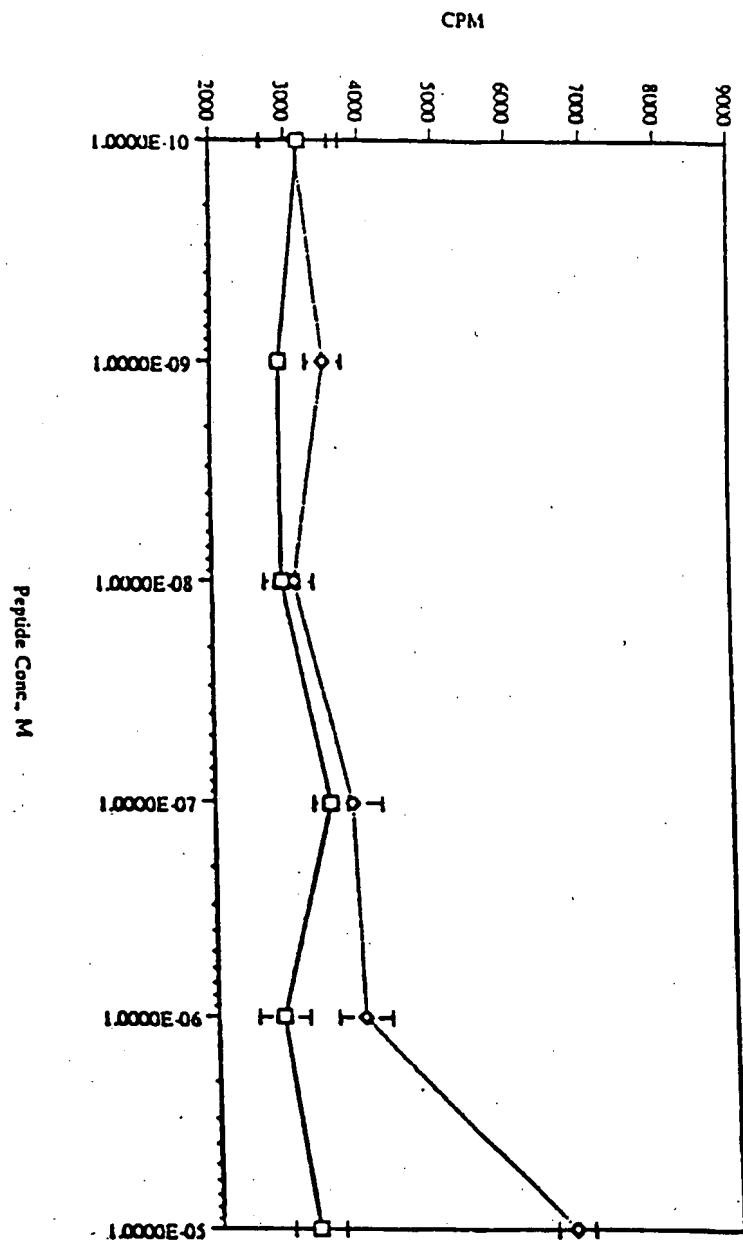


Figure 7



—□— Seq. I.D. No. 18  
—◇— Seq. I.D. No. 18D

Figure 8

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FIGURE 9

Page 1 of 3

Ac-GGTYSCHFGPLTWCKPQGG	SEQ ID NO: 20
GGTYRCSEMGFMTWVCLFNGG	SEQ ID NO: 21
GGTYSCFMGFMTWVCGFSGG	SEQ ID NO: 22
GGNAWCFMGFMTWVCSFNGG	SEQ ID NO: 23
GGNYSCFMGFMTWVCLFYGG	SEQ ID NO: 24
GGTYKCFMGFMTWVCKPFGG	SEQ ID NO: 25
GGDYTCFMGFMTWICTATGG	SEQ ID NO: 26
GGNYLCFPGFMTWCTGFRG	SEQ ID NO: 27
GGNYVCFMGFMTWICTPAGG	SEQ ID NO: 28
GGKDVCFMGFMTWICRSTGG	SEQ ID NO: 29
GGSYLCFMGFMTWLCIAQRGGG	SEQ ID NO: 30
GGNYLCFMGFMTWVCGRMGG	SEQ ID NO: 31
GGTYKCFMGFMTWVCGYAGG	SEQ ID NO: 32
GGDYTCFMGFMTWICTATRG	SEQ ID NO: 33
GGTYVCFMGFMTWICTATGG	SEQ ID NO: 34
GGTYSCFMGFMTWVCSFTGG	SEQ ID NO: 35
GGTYLCFMGFMTWVCERYGG	SEQ ID NO: 36
GGNYICRMGFMTWVCTAHGG	SEQ ID NO: 37
GGDYLCRMGFMTWVCGRMGG	SEQ ID NO: 38
GGLYLCRFGFMTWDCGYKGG	SEQ ID NO: 39
GGLYSCFMGFMTWVCTKAGG	SEQ ID NO: 40
GGGYHCRMGFMTWVCRPVGG	SEQ ID NO: 41
GGTYSCHFGPLTWCKPQGG	SEQ ID NO: 42
GGTYKCLMGFMTWVCTPDGG	SEQ ID NO: 43
GGLYSCLMGFMTWVCKPRGG	SEQ ID NO: 44
GGDYHCRMGFMTWVCKPLGG	SEQ ID NO: 45
GGDYSCRMGFMTWVCTPPGG	SEQ ID NO: 46
GGDYWCRMGFMTWVCHAHGG	SEQ ID NO: 47
GGKYLCSFGFMTWVCARYGG	SEQ ID NO: 48
GGLYKCLMGFMTWVCSPLGG	SEQ ID NO: 49
GGSYTCRFGFMTWVCRPMGG	SEQ ID NO: 50
GGSYSCRMGFMTWVCKPQGG	SEQ ID NO: 51
GGSYTCRMGFMTWVCLPAGG	SEQ ID NO: 52
GGLYECFMGFMTWVCRPQGG	SEQ ID NO: 53

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Figure 9  
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GGDYTCRMGPITWICTKAGG	SEQ ID NO: 54
GGVYSCRMGPITWECNRYVG	SEQ ID NO: 55
GGAYLCRMGPITWVCRPQGG	SEQ ID NO: 56
GGEYSCRMGPNTWVCKPVGG	SEQ ID NO: 57
GGLYLCRMGPVTWECOPRGG	SEQ ID NO: 58
GGLYTCRMGPITWVCLLPGG	SEQ ID NO: 59
GGLYTCRMGPVTWVCTGAGG	SEQ ID NO: 60
GGVYKCRMGPITWECRPTGG	SEQ ID NO: 61
GGDYNCRFGLTWCKPSSGG	SEQ ID NO: 62
GGSYLCRFGLTWLCSAGG	SEQ ID NO: 63
GGSYLCRMGPITWVCTPMGG	SEQ ID NO: 64
GGSYLCRFGLTWLCTQRGG	SEQ ID NO: 65
GGWVTCRMGPITWVCGVHGG	SEQ ID NO: 66
GGQLCGIGFITWVCRWVGG	SEQ ID NO: 67
GGXYSCFMGPITWVCSPVGRGV	SEQ ID NO: 68
GGWVYCRIGFITWVCDTHGG	SEQ ID NO: 69
GGMYTCRMGPMTWVCKGAGG	SEQ ID NO: 70
GGTTCQWIGFITWVCRARGG	SEQ ID NO: 71
GGPYHCRMGPITWVCGPVGG	SEQ ID NO: 72
GGEYTCRMGPISWVCSPOGG	SEQ ID NO: 73
GGNYTCRFGLTWECTPOGGGA	SEQ ID NO: 74
GGSWOCRIGFITWVCKHSGG	SEQ ID NO: 75
VGNMYHCRFGFITWVCRPGGG	SEQ ID NO: 76
GGLYLCRMGPQTMWCPGGG	SEQ ID NO: 77
GGDYVCRMGPMTWVCAFYGR	SEQ ID NO: 78
GGNYSCLMGPMTWVCKAJRG	SEQ ID NO: 79
GGKYTCRMGPMTWVCSFAGG	SEQ ID NO: 80
GGYVHCRIGFITWVCDIPGG	SEQ ID NO: 81
GSCLOCCIGFITWVCRHAGG	SEQ ID NO: 82
GGNYTCRMGPITWVCTPSSG	SEQ ID NO: 83
GGEYTCRMGPITWVCKRTGG	SEQ ID NO: 84
GGLYACRMGPITWVCKYHAG	SEQ ID NO: 85
GGQYLCFTGFITWLCRGAGG	SEQ ID NO: 86
GGVYACRMGPITWVCSPLGG	SEQ ID NO: 87
GGYTTTCRMGPITWVCSAHGG	SEQ ID NO: 88
GGTYKCRMGPMTWVCRPVGG	SEQ ID NO: 89
GGNYTCRFGLTWECNPTGG	SEQ ID NO: 90

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Figure 9  
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GGEYLCFNGPNTTCTFVGG	SEQ ID NO: 91
GGLYTCFNGPITTFCLPAGG	SEQ ID NO: 92
GGLYTCFNGPITTFCLPAGG	SEQ ID NO: 93